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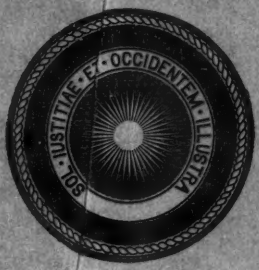

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ON THE DECOMPOSITION OF HEMICELLULOSES BY MICROÖRGANISMS: I. NATURE, OCCURRENCE, PREPARATION, AND DECOMPOSITION OF HEMICELLULOSES[1]

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In the decomposition of complex organic matter by microörganisms, many phenomena still remain obscure, largely because of an insufficient understanding of some of the chemical constituents of this organic matter and a lack of knowledge concerning the microörganisms bringing about the processes of decomposition. Only very recently has a simultaneous study been undertaken of the transformation of the various chemical constituents of plant residues in their decomposition by pure and mixed cultures of microörganisms (8, 30a, 90, 103). The results thus obtained have contributed to a better understanding of the decomposition processes that take place in manure and in soil, as well as to our knowledge of the origin of humus.

Of the various organic constituents in plant residues, attention has been in the past largely centered upon the decomposition of the sugars, celluloses, and proteins. Recently, the rôle of lignin in the degradation of organic matter under aerobic and anaerobic conditions has also attracted considerable attention, although very little is yet known concerning the microörganisms capable of bringing about the decomposition of this more or less resistant complex, and the mechanism of their action. Of the other major plant constituents, the hemicelluloses, a group of compounds second only to cellulose, as regards their relative abundance in plant tissues, have been studied only to a very limited extent, from the point of view of their decomposition by microörganisms. This was partly because of a lack of a clearer conception of the chemical nature of these compounds and partly because of an insufficient knowledge of the organisms concerned in their decomposition.

NOMENCLATURE AND COMPOSITION OF HEMICELLULOSES

The carbohydrates of plants are frequently classified into five groups: 1. Substances soluble in cold water; to this class being the mono-, di-, and trisaccharides, as well as certain water-soluble polysaccharides. 2. Carbohydrates insoluble in cold water, but yielding sugar under the action of diastase and similar enzymes; here belong starch, glycogen, dextrins, and inulin. 3.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

Carbohydrates insoluble in water and resistant to the action of the enzyme diastase, but soluble in alkalies and readily hydrolyzed by hot dilute mineral acids to give reducing sugars; this group comprises the heterogeneous hemicelluloses; the pectins and gums are related to this group, although they give on hydrolysis reducing sugars, in addition to other complexes. 4. Cellulose, sometimes referred to as true cellulose, which is hydrolyzed by concentrated mineral acids giving glucose only. 5. Lignins, a group of compounds, cyclic in nature; these complexes are not polysaccharides at all, but have been frequently classified with them because of the fact that they are associated together in the cell membrane of plants.

The term "hemicellulose" was first introduced by Schulze (98, 99). It was used to designate a group of substances entering into the composition of the cell membrane, similar to cellulose, but soluble in dilute alkali solutions and fairly readily hydrolyzed with dilute mineral acids, and yielding pentoses and hexoses. The function of hemicelluloses in plants is both of structural carbohydrates, similar to that of cellulose, and of reserve carbohydrates, similar to that of starch. The reserve hemicelluloses are found in kernels of seeds, especially those that are poor in starches and fats, the hexoses predominating as the products of hydrolysis. The structural hemicelluloses are found in seeds, fruit, husks, wood, straw, etc., and give, on hydrolysis, largely pentoses.

A number of various ill-defined compounds including the gums, certain slimy plant products, and various sugar condensation products are considered to be allied to hemicelluloses. The term "hemicellulose" has become so very confused and is so much misused, that it has recently been suggested (37, 41) that it be dropped altogether from scientific literature. Hess (37) preferred to designate this group of compounds as "cellulose associates," whereas Wise (129) suggested that they be referred to as "the non-cellulosic polysaccharides of the cell wall."

Recently the tendency has been (66, 70, 73, 74) to consider the hemicelluloses not as true polysaccharides, such as pentosans and hexosans, but as compounds containing considerable quantities of acid groups (glucuronic, galacturonic, mannuronic, etc., termed collectively "uronic acids"); the pectic substances may be considered as typical examples of such complexes. When pentosans are determined in plant materials by the "furfuraldehyde method" of Kröber (54), an error is frequently made in assuming that only the pentose units yield furfuraldehyde; the latter is obtained not only from the pentoses but also from the uronic acids.

$$\underset{\text{uronic acid}}{C_6H_{10}O_7} = \underset{\text{pentose}}{C_5H_{10}O_5} + CO_2$$

$$\underset{\text{pentose}}{C_5H_{10}O_5} = \underset{\text{furfural}}{C_5H_4O_2} + 3\ H_2O$$

Hemicelluloses, gums, and pectins are thus found to consist of pentoses, hexoses, and uronic acids, in varying proportions. Hemicelluloses from different

sources vary considerably in the products of hydrolysis, both qualitatively and quantitatively. O'Dwyer (74) found that beechwood contains two hemicelluloses, one of which yields on hydrolysis arabinose and galacturonic acid, and the other yields xylose and glucuronic acid. Norris and Preece (72) found four chemically and physically distinct hemicelluloses in wheat bran; two of these were true polysaccharides and two contained uronic acids. These and other (15, 65) investigations led Candlin and Schryver (13) to suggest the term "polyuronides" for complexes in which uronic acids are found in conjugation with sugars, largely pentoses. This term was supposed to include pectins and structural hemicelluloses which are very similar, while the term "hemicellulose" was reserved for substances which yield only sugars and no uronic acids on hydrolysis.

O'Dwyer (75) suggested a division of the hemicelluloses into A and B groups, on the basis of precipitation with glacial acetic acid and alcohol from the akaline extract; the nature of the hemicellulose was found to depend on the age of the plant and its nature. Norman (70) found that oat straw contained 22.8 per cent hemicellulose; the hemicellulose A consisted of 11 per cent uronic acid anhydride, 79 per cent arabinose and xylose calculated as anhydro-arabinose, and 10 per cent anhydro-galactose; whereas the hemicellulose B contained 32 per cent uronic acid anhydride and 68 per cent arabinose. Rye straw contained 33 per cent total hemicellulose, the A fraction comprising 5 per cent uronic acid anhydride, 60 per cent anhydro-pentose, and 35 per cent anhydro-hexose, whereas the B fraction was made up of about 29, 60, and 11 per cent of the respective three complexes. No fundamental difference was found (71a) to exist between the gums and the hemicelluloses.

For the present, before a more exact characterization and classification of these carbohydrates have been decided upon, we may refer to "hemicelluloses" as those polysaccharides which are insoluble in water or soluble with difficulty, but which are soluble in dilute alkalies and acids; they are readily hydrolyzed by hot dilute acids at ordinary pressure, giving on hydrolysis either pure monosaccharides (hexoses and pentoses), mixtures of monosaccharides, or a mixture of monosaccharides and uronic acids. These polysaccharides are not acted upon by diastase, inulase, or related enzymes, but some of them may be acted upon by certain special enzymes, such as cytases. The limits of this definition are evidently not very sharp. The hemicelluloses will thus comprise a wide variety of compounds, with pure polysaccharides related to starch and inulin at the one extreme and pure uronic acid complexes at the other; an illustration of the latter is found in algin or alginic acid, isolated from marine algae, which represents a polyuronic acid, in which all the carboxyl groups are free and all aldehyde groups conjugated (67). Most of the hemicelluloses will be found to contain both an acidic nucleus and a sugar fraction. The gums, mucilages, and pectins will thus be classified with the hemicelluloses; some of these substances, especially the pectins, may contain also other chemical groups, such as methyl alcohol and acetic acid.

OCCURRENCE OF HEMICELLULOSES IN NATURE

Hemicelluloses are widely distributed in the entire plant kingdom. They also occur abundantly in the bodies of microörganisms and in soil, having been introduced there by the plant residues or having been synthesized by the soil microbes. Hemicelluloses are particularly abundant in cell walls (58), in plant membranes (94), in root-stocks, rhizomes, and tubers (104), in wood (113), in cereal crops (86), and in a number of various seeds (16). These seeds usually contain also the corresponding hemicellulose-splitting enzymes or cytases; a number of higher animals were found to produce similar enzymes (88). Schulze (100) obtained from wheat and rye bran a hemicellulose which yielded arabinose and xylose on hydrolysis. Schulze and Pfenniger (101) later reported that the pods of *Pisum sativum* and *Phaseolus vulgaris* are rich in hemicelluloses, the content increasing with maturity of pods; on hydrolysis, galactose and arabinose were obtained. Indian clearing nuts were found (2) to contain a hemicellulose which gave on hydrolysis mannose and galactose, whereas that of ivory nut gave levulose and mannose. The hemicelluloses of American white oak (73) consist of 70 per cent pentosan (largely xylan, some araban) and 30 per cent hexosan, giving on hydrolysis mannose and galactose. The hemicelluloses or "gums" produced by bacteria are usually of the nature of compounds of various hexoses and pentoses with uronic acids (35). The literature dealing with the occurrence of hemicelluloses is most extensive, therefore no attempt will be made to review it here. Attention will be called to only certain specific hemicelluloses and their occurrence among plants, animals, and microörganisms.

Table 1 shows the relative abundance of hemicelluloses in fresh plant materials, in decomposed residues and in soil organic matter, as calculated from the reducing sugar produced on hydrolysis of the sugar and starch-free material with hot dilute hydrochloric acid.

Of the various individual hemicelluloses, the pentosans have received most consideration. This is no doubt both because of the considerably greater abundance of these carbohydrates in nature and because the determination of pentosans by the furfuraldehyde method is less complicated than in the case of the hexosans. Pentosans have been reported to be present in all parts of the apple tree and its fruit (19), in seeds (28), in the cotyledons of seeds (63), to a great extent among the sea weeds (64), and in lower (20), as well as higher fungi (126); they have also been reported to be present in soil (18). Agricultural crops, such as rice (48), corn fodder (82), wheat (108), and various other plants (112) contain pentosans as an integral part of their tissues. Extra precaution is needed to free the various forms of wood cellulose (44) from hemicelluloses. The furfuraldehyde method does not differentiate between the various pentosans. Even various uronic acids, such as glucuronic and galacturonic, which occur widely in plant products, will be reported by this method as pentosans. This is true especially of composts, peat and soils,

when nearly all the "pentosans" may be due to uronic acid complexes, as will be reported in detail later.

The distribution of the individual pentosans is almost as wide as that of the hemicellulose group as a whole. A marked increase in the pentosan content of

TABLE 1

Quantitative distribution of hemicelluloses in various plants, plant products, and soils

KIND OF MATERIAL	PER CENT OF TOTAL HEMICELLULOSE	REFERENCE
Alfalfa tops	13.14	118
Rye straw	26.41	120
Young corn stalks	20.38	118
Mature corn stalks	21.91	118
Young pine needles	14.68	118
Old pine needles	18.98	118
Green oak leaves	12.50	118
Mature oak leaves	15.60	118
Carex (upper growing portion)	18.36	124
Carex (rhizomes)	20.86	124
Hypnum	18.92	124
Sphagnum (upper growing portion)	30.82	124
Sphagnum (lower dead portion)	24.50	124
Fallen needles of *Pinus strobus*	18.98	124
Chestnut wood (sound)	15.23	122
Cypress wood (sound)	11.16	122
Non-fibrous decomposed wood	4.72	122
Fibrous decomposed wood	14.43	122
Fossilized wood from peat	8.15	122
Fossilized oak wood	3.79	122
Composted rye straw	14.77	120
Composted horse manure	12.67	120
Horse manure (fresh)	23.52	121
Cow manure (fresh)	18.57	121
Sheep manure (fresh)	18.46	121
Lowmoor peat (surface)	10.31	124
Lake peat	12.14	124
German highmoor peat:		
Younger sphagnum	16.88	124
Grenzhorizont	8.44	124
Older sphagnum	9.08	124
Woody peat	4.91	124
Pure wood from peat	5.06	124
Natural forest humus	15.28	125

the plant was found to take place with an increase in the maturity of the plant; the formation and accumulation of pentosan were thus observed to run parallel with those of the cellulose (117, 123). Schwalbe and Becker (102), however, reported that an increase in the lignin content of wood is accompanied by a

decrease in the pentosan content; they suggested that pentosan is polymerized to lignin. Similar observations were made by other investigators (24). Table 2 shows the relative abundance of pentosans in various plants and plant products, as calculated from the total yield of furfural, obtained on boiling with 12 per cent hydrochloric acid.

TABLE 2

The occurrence of pentosans in plants, plant products, and soil

KIND OF MATERIAL	PENTOSAN	REFERENCE
	per cent	
Ripe soy-beans	3.4	6
Green soy beans	3.6	6
Corn fodder	21.8	82
Corn hulls	48.6	83
Corn endosperm	1.7	83
Corn germ	8.4	83
Barley grain	12.6	116
Barley husks	6.5	116
Barley embryo	5.2	116
Rye straw	22.9	118
Timothy hay	22.0	121
Young leaves	5.3	16
Old leaves	9.7	16
Fungi (six varieties)	0.86–1.17	20
Wood fungi	1.20–6.50	126
Soils	0.05–2.50	103
Forest soil	0.75	25
Garden soil	0.39	25
Poor sandy soil	0.04	25
Lowmoor peat (surface horizon)	4.7	124
Forest peat (surface horizon)	0.75	124
Highmoor peat (surface horizon)	7.52	124
Nettles (bast fibers)	14.0	91
Fir	11.48	84
Pine	10.80	84
Birch	25.86	84
Poplar	22.71	84
Beech	24.30	84
Ash	23.68	84
Willow	23.31	84
Alder	22.94	84
Wood fissils	0–0.4	84
Brown coal (lignite)	0.3–0.4	84
Hard coal	0	84

The wood of dicotyledenous plants is very high in pentosan, containing 17 to 27 per cent of this type of hemicellulose, whereas the wood of gymnosperms contains only about 6 to 12 per cent pentosan and a considerable amount of

hexosan-hemicelluloses (17). König and Becker (52) have shown, for example, that the pentosan content of spruce (*Picea excelsa*) and of pine (*Pinus silvestris*) was 11.30 and 11.02 per cent respectively, whereas the pentosan content of beech (*Fagus silvatica*), birch (*Betula verrucosa*), and poplar (*Populus tremula*) was 24.86, 27.07, and 24.75 per cent respectively. The mature stalks of cereals are almost as rich in pentosans as are the various species of wood. Soils and peats contain comparatively low amounts of pentosans, as compared with the materials that enter into their formation. It is also of interest to note that lower filamentous fungi contain about 1 per cent pentosan, whereas higher fungi, such as the mushroom and wood destroying fungi may contain as much as 8.0 per cent. Rege (85) reported, for example, that *Coprinus* sp. separated from decomposing straw contained 7.82 per cent pentosan.

Xylans, methyl pentosans, and arabans usually occur together and closely associated in the natural state. Xylan is found to the greatest extent in wood (111). In some species of wood, xylan is the only hemicellulose present and can be isolated in an almost pure form with comparative ease, especially by the method proposed by Schmidt (94). *Fagus silvatica* was shown (95) to contain two xylans, one readily soluble and one soluble with greater difficulty. Straw (36) also contains large amounts of xylan, whereas plants like *Cucurbita pepo* (14) contain xylan in smaller amounts. Combinations of xylan and arabans have been observed in wood (73) and in sugar cane (10). In the latter case about four parts of xylan are found to every part of araban. In *Allium cepa* (33) xylan is found associated with a methyl pentosan. Xylans are commonly associated with glucosans, and arabans with galactans (101, 111). The xylans frequently form such a firm compound with cellulose as to be removed only by the action of strong acids or alkalies (94). The hemicelluloses of pine wood were found to consist of an easily and of a difficultly hydrolizable portion. The first comprised 18 per cent of the wood, and gave on hydrolysis, 17 per cent pentose, 42.7 per cent mannose, 4.2 per cent galactose, 3.2 per cent galacturonic acid, 4 per cent fructose, and 28.9 per cent glucose (32).

A methyl pentosan is a substance yielding methyl-furfural by distillation with 12 per cent HCl, in contrast to the furfural produced from the pentosans. Methyl pentosans occur in cereals (110), in onions (33), in the seeds of leguminous plants (63), in sea weeds (89), and are distributed in small amounts in a variety of plants (6, 57). Rice was found (48) to contain, on the average, 1 per cent methyl pentosan, and corn fodder, approximately 0.4 per cent (82).

Next to the pentosans, the mannans have received the widest study. Their general occurrence has attracted more attention than their relative abundance. It is to be regretted that not only the mannans, but also all the other hexosan-hemicelluloses, have been studied so little with respect to their quantitative occurrence. Mannans are found in asparagus seeds (12), in wood (73), in ivory nut, and in salep (36). In pine wood (106), a large amount of mannan is found in late summer and in early autumn. Sulfite pulp (38) contains considerable amounts of mannan. Various Oriental plants (114), algae, and sea

weeds (47, 49, 64, 77, 80, 87) are rich in mannans. The "mannans" as well are frequently preparations which give more than one sugar on hydrolysis, as in the case of the gluco-mannan, found in the leaves and fibers of *Amorphophallus konjac*, which gives glucose, mannose, and fructose, in varying ratios, such as 1:2:0, 2:5:0, or 2:3:1 (69a).

The galactans are neither as widely distributed (54) nor found in as large quantities as the pentosans. It has been shown conclusively that they are very abundant in sea weeds (3, 29, 53, 64, 93, 77). A substance isolated from Chinese moss (*Sphaerococcus lichenoides*) and originally called "gelose" (69) was found to be a galactan. In other cases, galactans have been found in the germinating embryos of carob, in *Nux vomica*, in fenugrec and lucerne (7), in onion (33), in cell walls of wood (58), in white oak (73), in western larch (97), in legumes (101, 56), and in fungi (21). From the quantitative standpoint, very small amounts are found in sugar cane (9) and as much as 13 per cent in *Abelmoschus Manihot* (79). Galacto-mannans have been found in date stones (36). Galacto-arabans were found in legumes, gum tragacanth, cherry and peat gums, in arabinic acid, etc. The function of galactose and galacturonic acid in the pectin molecule has been discussed in detail by Ehrlich (24).

Dextrans are synthesized by microörganisms as well as by higher plants. They have been reported in yeasts (59), in bacteria (61), and in fungi (127, 128). Certain species of sea weeds (64) contain dextrans associated with the other groups of hemicelluloses. The dextrans of wheat, rye, and barley have been divided into α- and β-amylans (1, 78). The α-amylans are soluble in hot water whereas cold water is sufficient to bring the β-amylans into solution. In *Asparagus officinalis* (12) the dextrans are combined with the mannans. Considerable quantities of hexosan-hemicelluloses have been found in wood, especially in evergreens (84). Various species of wood contain the following percentages of hexosans: fir, 13.58; pine, 12.78; birch, 4.61; poplar, 2.60; beech, 4.36; ash, 5.70; willow, 5.05; and alder, 22.94.

METHODS OF ANALYSIS OF HEMICELLULOSES

From what has been said before, one can readily recognize that the chemical structure of the hemicellulose group is not always definitely established. What knowledge there is of their structure does not even indicate whether a galactomannan is a polysaccharide built up of the residues of galactose and mannose or whether it is simply a mixture of galactans and mannans (84). Numerous investigations have shown that the hemicelluloses are associated with other complexes within the plant. In fact, a pure hemicellulose has never been found by itself in nature. Usually it is combined with the other complexes, but frequently it may simply occur alongside of them.

The methods of analysis of hemicelluloses are still purely conventional (39), as is shown by the lack of uniformity of the results. The usual procedure consists in hydrolyzing the material with dilute mineral acids (2 to 3 per cent) at boiling temperatures. Lower results are obtained by heating under pressure

at 118° to 125°C. with 1 per cent acid than by heating with 3 or 5 per cent acid at the temperature of boiling water. Different concentrations of acids, heating at different temperatures, and different periods of heating all cause variations in the amounts of hemicellulose obtained. The difficulties arise because substances of indefinite characters are being extracted and hydrolyzed. As will be shown later, the various hemicelluloses exhibit great differences in solubility and in resistance towards hydrolysis. Further, there may be other substances of a non-hemicellulosic character present which will yield the same end products upon hydrolysis. It becomes evident, therefore, that a method employed for determining hemicelluloses in all plants must be purely conventional. If accurate determinations of hemicelluloses are required, the plant complex in question containing the hemicelluloses must be studied thoroughly to determine the concentration of the acid to be used, the temperature to be employed, and the length of time for hydrolysis to take place.

Upon being heated with water (105), some hemicelluloses pass into a state of colloidal sols which are extremely viscous and revert to non-rigid gels after standing a few days. Their solutions yield, when evaporated, continuous films which possess a considerable tensil strength and are much tougher than films of nitrocellulose. As a rule, they are not acidic in character. The viscosity of these substances is increased by alkalies and boric acid, and decreased by heat, mineral acids, iron and copper salts, hydrogen peroxide, sodium peroxide, and potassium persulfate. They form adsorption compounds with the hydrates of calcium and barium. Heavy, solid, white, opaque gel formation takes place when basic lead acetate, potassium permanganate, or Fehling's solution is added. These viscous gels have such a high surface tension that water cannot penetrate. It is the hemicellulose content of the sea weeds that causes them to gel; agar-agar is a typical representative of that group of colloids (62); the addition of electrolytes lowers the swelling capacity of agar-agar.

The idea (70, 74) that hemicelluloses are not true carbohydrates in the strict chemical sense, wherein the proportion of hydrogen to oxygen must be the same as that of water, has been discussed in the foregoing. The two types of hemicelluloses found in beech wood led to this conclusion; upon hydrolysis, one yields xylose and an amount of carbon dioxide corresponding to 11 per cent of glucuronic acid, and the other yields arabinose and an amount of carbon dioxide corresponding to 63 per cent galacturonic acid. Similar results have been obtained in the case of straw. This view has been partially confirmed (72, 94).

The pentosan group is more specific than the general hemicellulose group. Many observations have been made concerning the chemical features of the furfural-producing substances, or the pentosans. The range of solubility of the pentosans is not narrow. Upon the basis of solubility they have been divided (22) as follows: (a) those soluble in water; (b) those soluble in 1 per cent hydrochloric acid solution; and (c) those soluble in 12 per cent hydrochloric acid. Of the pentosans in legumes (26), 44.50 per cent are dissolved

by 0.02 *N* acid and alkali solutions, 2.20 per cent are dissolved in 1.25 per cent sulfuric acid, and 26.70 per cent are dissolved by a 1.25 per cent alkali solution. The solubility of the pentosans of non-leguminous plants is quite similar to that of the pentosans of the legumes, but there is enough difference to give misleading results in some cases. In the non-leguminous plants, 24.80 per cent of the pentosans are dissolved by 0.02 *N* acid and alkali solutions, 26.90 per cent by a 1.25 per cent sulfuric acid solution, and 29.50 per cent by the alkali solution.

Hydrolysis of the pentosans with hot mineral acids may lead to the decomposition of some of the sugars, and one may not be able to account thus for all the pentosan. In studying the composition of pine wood (31), it was found that the H-ion concentration of the sulfite solution must be very carefully adjusted; if it is not of the proper concentration, the cellulose may be attacked, the pentosans may be decomposed, or both may occur. An acid mixture, composed of 80 gm. of sodium acid sulfate and 200 cc. of 0.1 *N* HCl made up to 1 liter with water, will dissolve all the pentosans without destroying the cellulose. When the acid concentration in this mixture is doubled, the cellulose is slightly attacked and some of the pentoses are decomposed. The glucose is not so readily decomposed. After pine wood has been heated in this stronger mixture at 100°C. for 4 days, 20 per cent of the glucose and 72 to 96 per cent of the various pentoses are decomposed.

Attempts to separate the hemicelluloses, the cellulose, and the lignin of plant material by various chemical treatments have not given accurate results. As an example, an analysis of sunflower seed hulls (46) may be cited. The hulls were extracted with ether, dilute hydrochloric acid, and ammonia, and the residue was treated successively with 5 per cent sodium hydroxide solution, Schweizer's reagent (ammoniacal copper hydroxide), dilute hydrochloric acid, and ammonia. This gave a product containing 2.78 per cent hemicellulose, 6.7 per cent cellulose, and 56.7 per cent lignin. In the various fractions obtained, the pentosans were found to make up a total of 81.4 per cent of the "hemicellulose" fraction, 54.5 per cent of the "cellulose" fraction, and 1.38 per cent of the "lignin" fraction. The products obtained on hydrolysis of plant material are intimately combined in the parent substance; these products should not be considered as absolutely identical with the actual plant constituents (9).

Various formulas have been submitted from time to time to explain the structure of pentosans. One of the oldest formulas (111) represented the pentosan as $(C_5H_8O_4)_n$. Within the last few years (96) the formula $(C_{10}H_{18}O_9)_n$ has been advanced. The basis for this formula lies in the fact that 100 per cent of pentosans can never be recovered when determined by the furfural-phloroglucide method, the purest preparations available yielding only 92 to 96 per cent pentosan; no other sugar except a pentose has ever been isolated from a pentosan.

The usual method of determination of pentosan consists in the distillation

with 12 per cent hydrochloric acid to form furfural; this is precipitated as furfural-phloroglucide by the addition of phloroglucin in a 12 per cent hydrochloric acid solution; the pentosan is then calculated from the amount of precipitate (81). These results are frequently too low because the hydrochloric acid may increase in concentration to about 18 per cent after boiling for some time and bring about the destruction of some of the furfural (115); or various aromatic compounds, especially the tannins, combine with the furfural and prevent it from being distilled over. On the other hand, the results obtained by this method may prove to be too high, since other substances besides pentoses yield furfural, especially the uronic acid derivatives. By determining the uronic acid content of the preparation, one can calculate the amount of furfural due to the uronic groups and the amount due to pentose groups (70).

It was found that the process could be hastened (5) by boiling the furfural-phloroglucide precipitate and filtering immediately rather than letting it stand over night. Since the methylpentosans yield methylfurfural upon distillation, the relative amounts of methylfurfural and furfural produced at various stages of the distillation have been studied (27). Most of the furfural is produced early in the distillation, whereas no methylfurfural is produced until at least 150 cc. has been distilled over. Furfural-phloroglucide and methylfurfural-phloroglucide may be separated on the basis of their solubility in ethyl alcohol, the latter being soluble (34). The formation of hydroxy-methylfurfural (76, 129) causes the results to be too high, as it cannot be separated by solubility in alcohol.

The furfural may also be precipitated with phenylhydrazine (60), fixed by a bisulfite solution or used to reduce copper compounds; it may also be titrated with a standard solution of potassium bromate (81); the formation of compounds with thiobarbituric acid was found (129) to give the most reliable quantitative results.

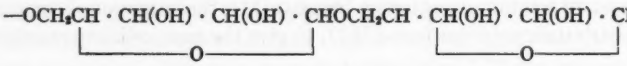
When pentosans are determined by means of hydrolysis with dilute mineral acids, some of the resulting pentoses may be destroyed (31) by the acid. It becomes necessary, therefore, to keep the H-ion concentration as low as possible, the time of reaction as short as possible, and to avoid high pressures, if accurate results are to be obtained.

It is sufficient to consider at further length the most common pentosan xylan, or "wood gum." Xylan is found abundantly in plant materials, especially in wood, in corn cobs, etc. Methyl-xylan has also been found in nature (42). When xylan is hydrolyzed with 12 per cent hydrochloric acid, 88 per cent of it is changed into sugar in 5 minutes' time, the maximum amount of pentose being formed in 5 to 10 minutes (39, 43). After 10 minutes, the amount of pentose decreases because of its conversion into furfural. When 5 per cent hydrochloric acid was used as the hydrolyzing agent, 90 per cent of the xylan was converted into xylose in 30 minutes. Sulfuric acid hydrolyzed xylan much more slowly than did hydrochloric acid, one hour being necessary to hydrolyze 85 per cent of the xylan with 12 per cent sulfuric acid. Continued action of

the aforementioned acids led to the formation of furfural and later its polymerization and decomposition. It has also been observed (41) that when xylan is heated with 45 parts of 3 per cent nitric acid, 84 per cent of it is hydrolyzed in one hour. This method has a certain advantage, since no furfural is formed in the hydrolytic process. The concentration of nitric acid given in the foregoing will completely dissolve the xylan in 1 to 2 minutes at 100°C. At this stage, part of it is probably in the xylo-dextrin stage. The use of nitric acid as a hydrolyzing agent causes the oxidation of the xylan molecule to tri-hydroxy-glutaric acid, and later to oxalic acid.

Xylan is resistant to the action of alkalies (45); it reacts with acetyl chloride to form xylan-monoacetate, an amorphous powder of the composition $C_5H_7O_2 \cdot C_2H_3O_2$ (111). The xylan-di-acetate is formed when it interacts with acetic anhydride. With benzoylchloride, in the presence of sodium hydroxide, xylan yields a xylan-monobenzoate. Concentrated nitric acid gives a xylan-nitrate. Xylan can be separated from other pentosans, especially from arabans and meta-arabans, by precipitation. Fehling's solution will precipitate all of the xylan from a sodium hydroxide solution (92), while most of the other pentosans remain in solution. In purifying xylan, it is difficult to remove all the free xylose from the xylan molecule proper.

Xylan has been called a polyose (50) in which a reducing group of one xylose molecule is joined with the hydroxyl group of another. The fact that xylan gives mono- and di-acetyl compounds upon acetylation indicates the presence of two free hydroxyl groups. Methylation, hydrolysis, and oxidation of the xylan molecule gave di-methoxy-glutaric acid (50), which led to the suggestion of the following structure for xylan molecule:



Schmidt (94, 94a) found that the residual substance obtained by treating plant material with ClO_2 and dilute sodium sulfite solution consists of cellulose and hemicellulose. The latter could be then dissolved out with sodium hydroxide solution and precipitated with alcohol and acid. Two xylans were thus obtained, one of which ("easily-soluble") was removed with dilute (0.04–0.2 per cent) NaOH solution and the other ("difficultly-soluble") was dissolved in more concentrated (5 per cent) NaOH solution.

Of the various other "hemicelluloses," the chemical structure of the pectins has received considerable attention. An extensive literature is available dealing with the chemical nature of this substance (7a). It is sufficient to mention here the fact that pectin of orange peels is considered (24) to consist of an araban and a Ca, Mg salt of pectic acid. The latter gives on hydrolysis several complexes,

$$\underset{\text{pectic acid}}{C_{41}H_{60}O_{36}} + 9\ H_2O = 4\ \underset{\text{galacturonic acid}}{C_6H_{10}O_7} + 2\ \underset{\text{methyl alcohol}}{CH_3OH} + 2\ \underset{\text{acetic acid}}{CH_3 \cdot COOH} + \underset{\text{arabinose}}{C_6H_{10}O_5} + \underset{\text{galactose}}{C_6H_{12}O_6}$$

Pectic acid is thus found to be a di-methoxy-di-acetyl-arabino-galacto-tetra-galacturonic acid.

PREPARATION OF CERTAIN CRUDE HEMICELLULOSES

In carrying out the following investigations on the decomposition of specific hemicelluloses by microörganisms, it was necessary to isolate and prepare these polysaccharides in a more or less pure form (107).

Dulse, a seaweed, was the source of the *pentosan* preparation. After thorough washing of the material with cold water, successive extracts with boiling water were made. The extracts were filtered and the filtrates thus obtained were concentrated on a water bath; the pentosan was precipitated from the solution by the addition of 3 volumes of 95 per cent ethyl alcohol. The precipitate was washed with 95 per cent ethyl alcohol and sulfuric ether, and then dried over sulfuric acid in a desiccator at a low temperature.

An analysis of the preparation thus obtained gave the following results:

	per cent
Moisture	17.40
Ash	7.00
Total nitrogen	0.57
Pentosan	38.34
Total hemicellulose	60.00

The moisture content was determined by drying to constant weight in an electric oven at 105°, and the ash by ignition. The ordinary Kjeldahl method was used for the determination of total nitrogen. The pentosan was calculated from the furfural produced by distillation of the material with 12 per cent HCl; the furfural was precipitated with phloroglucin. Hydrolysis with 2 per cent hydrochloric acid solution and determination of the resulting reducing sugars gave the total amount of hemicellulose.

The preparation thus obtained was insoluble in cold and hot water. A very fine suspension could be made in hot water; it required considerable time for it to precipitate out again. The preparation did not reduce Fehling's solution. At the boiling point with 2 per cent HCl it went into solution and could then be easily hydrolyzed to simple reducing sugars. It had practically no swelling capacity in water. The ash content of this preparation was noticeably high and the crude protein made up a little over 3 per cent of the total constituents. Although this was called a pentosan preparation, only about 65 per cent of the total hemicellulose was found to be pentosan.

The *mannan* was extracted from salep roots. It was brought into solution with hot water, after thorough washing with cold water. Since the solution could not be filtered because of its viscous nature, the solid particles were centrifuged from the solution. The mannan was then precipitated with 95 per cent ethyl alcohol and the precipitate dried as in the case of the pentosan preparation.

Upon analysis, it gave the following composition:

	per cent
Moisture	22.90
Ash	1.40
Total nitrogen	0.14
Pentosan	1.82
Total hemicellulose	57.48

This preparation was not soluble in cold water and did not reduce Fehling's solution. When it was allowed to become fully imbibed with distilled water, it swelled to three times its dry volume. The ash and total nitrogen contents of this preparation were very low. Only a small portion of the hemicellulose was in the form of pentosan. The reaction with phenylhydrazine showed that the preparation consisted principally of mannan.

The *galactan* was extracted from Irish moss (*Chondrus crispus*), in a manner similar to the above procedures, giving a preparation which analyzed as follows:[2]

	per cent
Moisture	38.60
Ash	18.00
Total nitrogen	0.44
Pentosan	2.18
Total hemicellulose	31.64

It was difficult to remove the residual moisture of this material. This preparation may appear to be low in hemicelluloses, but when the ash and moisture are considered, it becomes obvious that it was as pure as either of the preceding preparations. It was not soluble in cold water and did not reduce Fehling's solution. Upon wetting, it swelled to two and one-half times its dry volume. Haas (30b) concluded that the ash of carragen (*Chondrus crispus*), which cannot be reduced by dialysis and which consists principally of calcium and sulfate, is an integral part of the carragen molecule and is present there as a sulfuric ester.

The *xylan* was extracted from corn cobs. The method used was that of Salkowski as modified by Heuser (84). It consists in extracting the xylan with 5 per cent NaOH solution under 15 pounds pressure for 1 hour and precipitating the xylan with Fehling's solution. The xylan was freed from copper by washing with alcohol saturated with HCl gas. The copper-free compound was washed with ethyl alcohol and ether, and dried in a desiccator over sulfuric acid at a low temperature.

The analysis of the preparation gave the following results:

	per cent
Moisture	5.7
Ash	6.9
Total nitrogen	0.0
Pentosan	88.3
Total hemicellulose	87.7

[2] More purified preparations were later obtained, containing 9.45 per cent moisture and 21.00 per cent ash.

This was by far the purest preparation obtained. Calculated upon the ash- and moisture-free basis, it was made up of 96 per cent pentosan. The reaction with phenyl hydrazine showed that the substance is built up only of xylose anhydrides. It was readily soluble in hot HCl, insoluble in cold water, and only slightly soluble in hot water. It did not reduce Fehling's solution. Upon becoming fully imbibed with distilled water, it attained a volume of approximately 1.5 times its original dry volume. It did not form a gummy mass when placed in cold water, a characteristic property of the aforementioned hexosans studied.

These crude preparations were used in the first studies of decomposition of hemicelluloses by microörganisms, which will be reported in the following contributions. The last three preparations were practically free from uronic acid complexes, hence they can be considered as true hemicelluloses and not as polyuronides.

DECOMPOSITION OF HEMICELLULOSES IN PEAT, IN SOIL, AND IN COMPOSTS

In most of the former investigations on the decomposition of pentosans or of hemicelluloses in general, no attempt has been made to obtain a preparation free from sugars, proteins, cellulose, and lignin. The utilization of hemicellulose by microörganisms was measured by determining its content in the natural material at the beginning and at the end of the decomposition period. It was found, for example (23), that when manure undergoes decomposition, the pentosans are attacked more quickly than the cellulose, whereas toward the end of the decomposition period, more pentosan is left in the compost than in the cellulose. In general pentosans are among those substances which are actively decomposed in the manure pile (121). When sheep manure was allowed to decompose with 100, 200, and 400 per cents moisture, the amounts of hemicellulose left at the end of 192 days were 9.14, 7.31, and 11.62 per cent respectively, compared with 18.46 per cent in the original manure. These results have shown that there is an optimum moisture for the decomposition of the hemicelluloses. Fresh horse manure, on a dry basis, contained 23.52 per cent hemicellulose. When allowed to decompose, the percentage of hemicellulose found after an incubation period of 39, 96, 156, and 290 days, was 22.84, 15.76, 13.36, and 12.67 respectively. When one keeps in mind the fact that a considerable reduction in the weight of dry material has taken place as a result of decomposition, one will readily recognize the rapidity with which these hemicelluloses decompose in compost.

The nature and age of the plant residues have considerable influence upon the rapidity with which the hemicelluloses disappear in the process of decomposition. In the case of rye plants, for example, the rate of decomposition of the pentosans was inversely proportional to the age of the plant (123). In the case of mature rye straw undergoing decomposition in a compost, 77.7 per cent of the pentosans disappeared in two months' time (120), in the presence of available minerals and at an optimum moisture content. The fact that

available nitrogen is necessary in order that the fungi and bacteria can decompose the hemicelluloses was well illustrated. Without additional inorganic nutrients, 15.2 per cent of the hemicellulose was decomposed in 28 days, whereas with added nutrients, the amount decomposed during the same period was 64.7 per cent of the total. In the case of semimature corn stalks, the amounts of hemicelluloses decomposed during the same period in the composts, without and with inorganic nutrient salts were 50.6 per cent and 81.4 per cent respectively. Mature rye straw composts containing 2,139 gm. of hemicellulose lost the following amounts within a period of 290 days: untreated compost, 868.3 gm.; calcium carbonate added, 706.2 gm.; diammonium phosphate and potassium chloride added, 1,407.6 gm.; a combination of the three salts, 1565.5 gm.

In the composting of oak leaves under aerobic and anaerobic conditions (125), in the presence of diammonium phosphate and calcium carbonate, large quantities of hemicellulose were decomposed, as is shown in table 3.

TABLE 3

Decomposition of oak leaves under aerobic and anaerobic conditions

CONDITIONS	AMOUNTS OF HEMICELLULOSE PRESENT				
	Original leaves	After 6 months		After 12 months	
		No $CaCO_3$	With $CaCO_3$	No Ca	With Ca
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Aerobic	25.00	6.62	6.62	5.85	5.55
Anaerobic	25.00	22.95	22.72	12.81	7.45

According to Rege (85), two factors influence the rapidity of decomposition of plant material, in the presence of sufficient available nitrogen: the "energy" factor, which he associated with the pentosans, and the "inhibitory" factor which he believed to be the lignin fraction; he suggested that the ratio between these two factors can enable one to predict the rapidity of decomposition of the given material. This assumption is totally unjustified, as shown recently by Norman (71) and previously by Egorov (24) and in this laboratory (109), where the hemicelluloses were found to decompose rapidly during the first few days, and later only slowly.

This was found to be due to three factors: (a) the non-homogeneous nature of the hemicelluloses, some of which are decomposed much more readily than others (b) the possible production of some intermediate substance which yields furfuraldehyde (70), and (c) the synthesis of new hemicelluloses by microorganisms. The reason for the second assumption was found (71) in the small loss of uronic acid in the process of decomposition of straw. The third factor was established in former contributions from this laboratory on the synthesizing activities of microörganisms.

This phenomenon can further be illustrated when one compares the relative hemicellulose and cellulose content of a lowmoor peat (119, 124); although the plants from which the peat originated contained a much higher cellulose than hemicellulose content, the peat itself was practically free from cellulose, but it still contained 6 to 12 per cent hemicellulose. von Feilitzen and Tollens (25) found that the pentosan content of peat diminishes with depth, 6.26 to 12.75 per cent of pentosan being found in the surface layers. Since more hemicellulose was decomposed in the older peat horizons, an attempt has been made to determine the age of the horizon by the amount of hemicelluloses present (115). The same is true of the organic matter layer in forest soils, which may contain 12 to 18 per cent hemicellulose and only 2.5 to 10 per cent cellulose, whereas the reverse ratio between these two plant constituents is found in the undecomposed plant residues (125).

König and associates (51) have shown that the hemicelluloses added to the soil in the manure decompose more readily than the total carbon complexes (due to the resistance of the lignin) and, after a period of one year, nearly all the pentosans have disappeared. At least some of the hemicelluloses in the organic matter of mineral soils is of microbial origin (30).

Various fungi (69) and bacteria (4) are capable of producing cytases or enzymes capable of hydrolyzing hemicelluloses. A detailed discussion of the decomposition of various hemicelluloses by pure cultures of microörganisms will be presented later.

SUMMARY

1. Attention is called to the confused state of our present knowledge of the hemicelluloses, which form an important group of chemical constituents of plants and microörganisms.

2. The chemistry of hemicelluloses, methods of analysis, and the occurrence of these carbohydrates in nature are discussed in detail.

3. A general review is presented of the decomposition of hemicelluloses by microörganisms, when plant materials undergo decomposition in manure compost and in soil.

REFERENCES

(1) Abderhalden, E. 1911 Hemicellulosen (by V. Grafe). *Biochem. Handlex.* 2: 42–65.

(2) Baker, J. L., and Pope, T. H. 1900 Mannogalactan and laevulomannan. Two new polysaccharides. *Jour. Chem. Soc.* 77: 696–705.

(3) Bauer, R. W. 1884 Ueber den aus Agar-agar entstehenden Zucker, über eine neue Säure aus der Arabinose nebst dem Versuch einer Classification der gallertbildenden Kohlehydrate nach den aus ihnen entstehenden Zuckerarten. *Jour. Prakt. Chem.* (N. F.) 30: 367–388.

(4) Biernacki, W. 1911 *Bacterium Nenckii* Biern., ein neuer den Agar verflüssigender Mikroorganismus. *Centbl. Bakt.* (II) 29: 166–169.

(5) Böddener, K. H., and Tollens, B. 1910 Über eine Modifikation der Furfurol-Salzsäure-Bestimmungsmethode der Pentosane. *Jour. Landw.* 58: 232–237.

(6) BORGHESANI, G. 1910 Über das Verhältnis der Methylpentosane gegenüber den Pentosanen in einigen Arten von Pflanzensamen. *Jour. Landw.* 58: 77–79.

(7) BOURQUELOT, E., AND HÉRISSEY, H. 1899 Germination de la graine de Caroubier; production de mannose par un ferment soluble. *Compt. Rend. Acad. Sci.* (Paris) 129: 614–616.

(7a) BRANFOOT, M. H. (M. H. Carré) 1929 A critical and historical study of the pectic substances of plants. *Dept. Sci. Ind. Res. London Sp. Rpt.* 33.

(8) BRAY, M. W., AND ANDREWS, T. M. 1924 Chemical changes of groundwood during decay. *Jour. Indus. Engin. Chem.* 16: 137–139.

(9) BROWNE, C. A. 1904 The hydrolytic products of sugar-cane fiber. *Jour. Amer. Chem. Soc.* 26: 1221–1235.

(10) BROWNE, C. A. 1904 Results of investigations at the Sugar Experiment Station laboratory for 1903. *La. Planter* 32: 4751.

(11) BUTLER, C. L., AND CRETCHER, L. H. 1929 The composition of gum arabic. *Jour. Amer. Chem. Soc.* 51: 1519–1525.

(12) CAKE, L. S. E., AND BARTLETT, H. H. 1922 The carbohydrate content of the seed of *Asparagus officinalis*. *Jour. Biol. Chem.* 51: 93–102.

(13) CANDLIN, E. J., AND SCHRYVER, S. B. 1928 Investigations of the cell-wall substances of plants, with special reference to the chemical changes taking place during lignification. *Proc. Roy. Soc. London.* (B) 103: 365–376.

(14) CASTORO, N. 1907 Über die in den Samenschalen von *Cucurbita Pepo* enthaltenden Hemicellulosen. *Ztschr. physiol. Chem.* 52: 521–525.

(15) CLAYSON, D. H. F., NORRIS, F. W., AND SCHRYVER, S. B. 1921 Pectic substances of plants: II: A preliminary investigation of the chemistry of the cell walls of plants. *Biochem. Jour.* 15: 643–653.

(16) CZAPEK, F. 1913–1921 Biochemie der Pflanzen, ed. 2. G. Fischer, Jena.

(17) DECHALMOT, G. 1893, 1894 Pentosans in plants. *Amer. Chem. Jour.* 15: 21–38, 276–285 (1893); 218–228, 589–611 (1894).

(18) DECHALMOT, G. 1894 Pentosans in soils. *Amer. Chem. Jour.* 16: 239.

(19) DELONG, W. A. 1928 Pentosan content in relation to winter hardiness in the apple. *Sci. Agr.* 8: 501–523.

(20) DOX, A. W., AND NEIDIG, R. E. 1911 Pentosans in lower fungi. *Jour. Biol. Chem.* 9: 267–269.

(21) DOX, A. W., AND NEIDIG, R. E. 1914 The soluble polysaccharides of lower fungi: II. Mycogalactan, a new polysaccharide in *Aspergillus niger*. *Jour. Biol. Chem.* 19: 235–237.

(22) DOYLE, J., AND CLINCH, P. 1926 Pentosan theory of cold-resistance applied to conifers. *Sci. Proc. Roy. Dublin Soc.* 18: 219–235.

(23) EGOROV, M. 1912 Report on experiments on the decomposition of horse manure. *Ann. Inst. Agron. Moscow* 17: 1–58.

(24) EHRLICH, F. 1930 Ueber die Chemie des Pektins und seine Beziehung zur Bildung der Inkrusten der Cellulose. *Cellulosechemie* 11: 140–151, 161–170.

(25) FEILITZEN, H., VON, AND TOLLENS, B. 1898 Über den Gehalt des Torfes an Pentosan und andern Kohlenhydraten. *Jour. Landw.* 46: 17–22.

(26) FRAPS, G. S. 1915 Distribution and digestibility of pentosans of feeds. *Tex. Agr. Exp. Sta. Bul.* 175: 5–24.

(27) GIERISCH, W. 1925 Determination of pentosans in wood. *Cellulosechemie.* 6: 61–74, 81–93.

(28) GOY, S. 1912 Über Pentosane. *Fühlings Landw. Zeit.* 61: 606–612.

(29) GREENISH, H. 1881 Untersuchung von *Fucus amylaceous*. *Ber. deut. Chem. Gesell.* 14: 2253.

(30) GREIG-SMITH, R. 1912 Bacterial slimes in soil. *Proc. Linn. Soc. N. S. Wales* 46: 609–612.

(30a) GROSZKOPF, W. 1929 Über das stoffliche und morphologische Verhalten lignin-reicher Nadelholzgewebe bei der Bildung von Waldhumus und Braunkohle. *Brennstoff-Chem.* 10: 161–167, 213–217.

(30b) HAAS, P. 1921 Carragen (*Chondrus crispus*). II. The occurrence of ethereal sulfates in the plant. *Biochem. Jour.* 15: 469–476.

(31) HÄGGLUND, E. 1928 Holzchemie. Akad. Verlagsges, Leipzig.

(32) HÄGGLUND, E., KLINGSTEDT, F. W., ROSENQUIST, T., AND URBAN, H. 1928 Über die Kohlenhydratbestandteile der leicht hydrolysierbaren Hemicellulose von Fichte. *Ztschr. Physiol. Chem.* 177: 248–263.

(33) HASHITANI, Y. 1924 The hemicellulose of *Allium capa L.* *Jour. Coll. Agr. Hokkaido Imp. Univ.* 14: 57–63.

(34) HAYWOOD, J. K. 1907 Report on foods and feeding stuffs. *U. S. Dept. Agr. Bur. Chem. Bul.* 105: 112–116.

(35) HEIDELBERGER, M., AND GOEBEL, W. F. 1927 The soluble specific substance of Friedländers bacillus. *Jour. Biol. Chem.* 74: 613–618, 619–629.

(36) HERZOG, R. O., AND GONELL, H. 1924 Weitere Untersuchungen an Naturstoffen und biologischen Strukturen mittels Röntgenstrahlen. *Naturwissenschaften* 12: 1153–1155.

(37) HESS, K. 1928 Die Chemie der Zellulose und ihrer Begleiter. Akad. Verlagsges, Leipzig.

(38) HESS, K., AND LÜDTKE, M. 1928 Isolierung von Mannan und Xylan aus Sulfitzellstoff. *Liebigs Ann.* 466: 18–26.

(39) HEUSER, E. 1923 Ueber den Pentosangehalt des Lignins. *Cellulosechemie* 4: 77–78.

(40) HEUSER, E. 1927 Hemicellulosen. *Papierfabr.* 25: (Tech. Wiss. Teil) 238–243.

(41) HEUSER, E. 1927 Lehrbuch der Cellulosechemie, Ed. 3. Berlin.

(42) HEUSER, E., AND BRADEN, M. 1921, 1922 Beiträge zur Kenntnis der Pentosane: I. Die Darstellung von Xylan. *Jour. Prakt. Chem.* 103: 69–75 (1921); 104: 259–264 (1922).

(43) HEUSER, E., AND BRUNNER, L. 1922, 1923 Beiträge zur Kenntnis der Pentosane: IV. Hydrolyse des Xylans mit verdünnten Säuren. *Jour. Prakt. Chem.* 104: 264–281 (1922); 105: 232–241 (1923).

(44) HEUSER, E., AND DAMMEL, W. 1924 Ueber den Mannan-Gehalt von Zellstoff und seine analytische Bestimmung. *Cellulosechemie* 5: 45–53.

(45) HEUSER, E., AND ROTH, J. 1924 Beiträge zur Kenntnis der Pentosane. Die Kalischmelze des Xylans. *Jour. Prakt. Chem.* 107: 1–6.

(46) HOFFMEISTER, W. 1898 Die quantitative Trennung von Hemicellulose, Cellulose und Lignin und das Vorkommen der Pentosane in diesen. *Landw. Vers. Sta.* 50: 347–362.

(47) ISHII, J. 1894 Mannane as a reserve material in the seeds of *Diospyros kaki. Col. Agr. Tokyo, Japan, Bul.* 2: 101–102.

(48) KATAYAMA, T. 1915 Pentosans contained in rice. *Jour. Chem. Indus. Japan* 18: 1364.

(49) KINOSHITA, Y. 1895 On the occurrence of two forms of mannan in the root of *Conophallus konjaku. Col. Agr. Tokyo, Japan, Bul.* 2: 205.

(50) KOMATSU, S., AND KASHIMA, K. 1922 Constitution of the polysaccharides: I. Xylan and its acetyl derivatives. *Mem. Col. Sci. Kyoto Univ.* 5: 307–314.

(51) KÖNIG, J., BACH, M., BALKS, R., AND HASENBÄUMER, J. 1926 Die Zersetzung des Stalldüngers im Boden und seine Ausnutzung durch die Pflanzen. *Mitt. Deut. Landw. Gesell.* H. 26–27.

(52) KÖNIG, J., AND BECKER, E. 1919 Die Bestandteile des Holzes und ihre wirtschaftliche Verwertung. *Ztschr. Angew. Chem.* 32: 155–160.

(53) KÖNIG, J., AND BETTELS, J. 1905 Die Kohlenhydrate der Meeresalgen und daraus hergestellter Erzeugnisse. *Ztschr. Untersuch. Nahr. u. Genussmtl.* 10: 457–473.

(54) KRÖBER, E. 1900 Untersuchungen über die Pentosanbestimmungen mittelst der Salzsäure-Phloroglucinmethode nebst einigen Anwendungen. *Jour. Landw.* 48: 357–384.

(55) LINDSEY, J. B., AND HOLLAND, E. B. 1897 The distribution of galactan. *Ninth Ann. Rpt. Mass. Hatch Exp. Sta.* 1897: 92–96.

(56) MAXWELL, W. 1890 The presence of sugar-yielding insoluble carbohydrates in seeds. *Amer. Chem. Jour.* 12: 51–60.

(57) MAYER, W., AND TOLLENS, B. 1907 Über die quantitative Bestimmung der Fukose und der Methyl-pentosane. *Ber. Deut. Chem. Gesell.* 40: 2441–2442.

(58) MEHTA, M. M. 1925 Biochemical and histological studies of lignification: II. Histological studies of the polysaccharides and aromatic constituents of the cell wall. *Biochem. Jour.* 19: 958–978.

(59) MEIGEN, W., AND SPRENG, A. 1908 Ueber die Kohlehydrate der Hefe. *Ztschr. Physiol. Chem.* 55: 49–73.

(60) MENAUL, P., AND DOWELL, C. T. 1919 A modification of the phenyl-hydrazine method of determining pentosans. *Jour. Indus. Eng. Chem.* 11: 1024–1025.

(61) MEYER, A. 1900 Über Geisseln, Reservestoffe, Kerne and Sporenbildung der Bakterien. *Chem. Centbl.* 2: 56–57.

(62) MICHAELIS, L. 1923 The effect of ions upon colloidal systems. Williams and Wilkins Co., Baltimore, Md.

(63) MIYAKE, K. 1912 The behavior of pentosan and methyl-pentosan of the seeds of *Glycine hispida* and of *Phaseolus vulgaris* during germination. *Jour. Col. Agr. Tohoku Imp. Univ. Japan* 4: 327–335.

(64) MÜLLER, K. 1905 Die chemische Zusammensetzung der Zellmembranen bei verschiedenen Kryptogamen. *Ztschr. Physiol. Chem.* 45: 265–298.

(65) NANJI, D. R., PAYTON, F. J., AND LING, A. R. 1925 Decarboxylation of polysaccharide acids: its application to the establishment of the constitution of pectins and to their determination. *Jour. Soc. Chem. Indus.* 44: 253T–258T.

(66) NANJI, D. R., AND NORMAN, A. G. 1928 Studies on pectin: II. The estimation of the individual pectic substances in nature. *Biochem. Jour.* 22: 596–604.

(67) NELSON, W. L., AND CRETCHER, L. H. 1929 The alginic acid from *Macrocystis pyrifera*. *Jour. Amer. Chem. Soc.* 51: 1914–1922.

(68) NEWCOMBE, F. C. 1898 Cellulose-enzymes. *Ann. Bot.* 13: 49–81.

(69) NIEBLING, R. 1890 Untersuchungen über die künstliche Verdauung landwirtschaftlicher Futtermittel nach Stutzer und Pepsinwirkungen im Allgemeinen. *Landw. Jahrb.* 19: 149–188.

(69a) NISHIDA, K., AND HASHIMA, H. 1930 Chemische Untersuchungen ueber das Glukomannan aus Konjak. *Jour. Dept. Agr. Kyushu Imp. Univ.* 2: 277–360.

(70) NORMAN, A. G. 1929 The biological decomposition of plant materials: I. The nature and quantity of the furfuraldehyde-yielding substances in straws. *Biochem. Jour.* 23: 1353–1366.

(71) NORMAN, A. G. 1929 The biological decomposition of plant materials: II. The rôle of the furfuraldehyde-yielding substances in the decomposition of straws. *Biochem. Jour.* 23: 1367–1384.

(71a) NORMAN, A. G. 1929 The chemical nature of the gums: I. The nature of the gum arabic and the biochemical classification of the gums. *Jour. Agri. Sci.* 23: 524–535.

(72) NORRIS, F. W., AND PREECE, I. A. 1930 Studies on hemicelluloses: I. The hemicelluloses of wheat bran. *Biochem. Jour.* 24: 59–66.

(73) O'DWYER, M. H. 1923 The hemicelluloses: III. The hemicellulose of American white oak. *Biochem. Jour.* 17: 501–509.

(74) O'DWYER, M. H. 1926 The hemicelluloses: IV. The hemicelluloses of beech wood. *Biochem. Jour.* 20: 656–664.

(75) O'Dwyer, M. H. 1928 Preliminary investigation on the constitution of the hemicelluloses of timber. *Biochem. Jour.* 22: 381–390.

(76) Oshima, K., and Kondo, K. 1926 The detection of methyl-pentosan. *Jour. Col. Agr. Hokkaido Imp. Univ.* 16: 1–11.

(77) Oshima, K., and Tollens, B. 1901 Ueber das Nori aus Japan. *Ber. Deut. Chem. Gesell.* 34: 1422–1424.

(78) O'Sullivan, C. 1881 On α- and β-amylan. *Chem. News* 44: 258.

(79) Ozawa, T. 1922 Mucilage of Abelmoschus Manihot. *Jour. Chem. Indus. (Japan)* 25: 389–405.

(80) Paton, F. J., Nanji, D. R., and Ling, A. R. 1924 The hydrolysis of the endosperm of *Phytelephas macrocarpo* by its own enzymes. *Biochem. Jour.* 18: 451–454.

(81) Pervier, N. C., and Gortner, R. A. 1923 The estimation of pentoses and pentosans: I. The formation and distillation of furfural. *Jour. Indus. and Engin. Chem.* 15: 1167–1169, 1255–1262.

(82) Peterson, W. H., Fred, E. B., and Verhulst, J. H. 1921 The destruction of pentosans in the formation of silage. *Jour. Biol. Chem.* 46: 329–338.

(83) Porst, C. E. G. 1912 Occurrence of pentosans and hydrolyzed pentosan in corn and corn products. *8th Internatl. Cong. Appl. Chem. Sec.* 6a: 205–212.

(84) Pringsheim, H. 1923 Die Polysaccharide, p. 183–193. Berlin.

(85) Rege, R. D. 1927 Biochemical decomposition of cellulosic materials, with special reference to the action of fungi. *Ann. Appl. Biol.* 14: 1–44.

(86) Reinitzer, F. 1897 Ueber das zellwandlosende Enzym der Gerste. *Ztschr. Physiol. Chem.* 23: 175–209.

(87) Reiss, R. 1889 Ueber die Natur der Reservecellulose und über ihre Auflösungsweise bei der Keimung der Samen. *Ber. Deut. Bot. Gesell.* 7: 322–329.

(88) Rippel, A. 1921 Das Vorkommen hemizellulosespaltender Enzyme in ruhenden Samen und die angebliche Lösung von Hemizellulosen durch Enzyme höherer Tiere. *Landw. Vers. Sta.* 97: 179–193.

(89) Rohmann, F. 1899 Einige Beobachtungen uber die Verdauung der Kohlehydrate bei Aplysien. *Centbl. Physiol.* 13: 455.

(90) Rose, R. E., and Lisse, W. M. 1917 The chemistry of wood decay. *Jour. Indus. and Engin. Chem.* 9: 284–287.

(91) Routala, O., and Sevon, J. 1927 Untersuchungen über die Nesselcellulose. *Cellulosechemie.* 8: 16–18.

(92) Salkowski, E. 1901–1902. Ueber die Darstellung des Xylans. *Ztschr. Physiol. Chem.* 34: 162–180.

(93) Schmidt, C. 1844 Ueber Pflanzenschleim und Bassorin. *Leibigs Annal. Chem.* 51: 56.

(94) Schmidt, E., and Graumann, E. 1921, 1927, 1929 Zur Kenntnis pflanzlicher Incrusten. *Ber. Deut. Chem. Gesell.* 54: 1860–1873 (1921); 60: 503–526 (1927); *Cellulosechemie* 10: 126–134 (1929).

(95) Schmidt, E., Meinel, K., Nevros, K., and Jandebeur, W. 1930 Ueber die ganzzahlige Beziehung der Cellulose zu dem s. l. Xylan in der Skelettsubstanz der Rotbuche (*Fagus silvatica*). *Cellulosechemie* 11: 49–68.

(96) Schorger, A. W. 1926 The chemistry of Cellulose and Wood, p. 144–174. McGraw Hill Book Co., New York.

(97) Schorger, A. W., and Smith, D. F. 1916 The galactan of *Larix occidentalis*. *Jour. Indus. and Engin. Chem.* 8: 494–499.

(98) Schulze, E. 1891 Zur Kenntniss der chemischen Zusammensetzung der pflanzlichen Zellmembranen. *Ber. Deut. Chem. Gesell.* 24: 2277–2287.

(99) Schulze, E. 1892 Zur Chemie der pflanzlichen Zellmembranen. *Ztschr. Physiol. Chem.* 16: 387–428.

(100) SCHULZE, E. 1894 Zur Chemie der pflanzlichen Zellmembranen. *Ztschr. Physiol. Chem.* 19: 38–69.

(101) SCHULZE, E., AND PFENNINGER, U. 1910 Uber das Vorkommen von Hemicellulosen in den Samenhülsen von *Pisum sativum* und von *Phaseolus vulgaris*. *Ztschr. Physiol. Chem.* 68: 93–108.

(102) SCHWALBE, C. G., AND BECKER, E. 1920 Die chemische zusammensetzung des Erlenholzes. *Ztschr. Angew. Chem.* 33: 14–16.

(103) SHOREY, E. C., AND LATHROP, E. C. 1910 Pentosans in soils. *Jour. Amer. Chem. Soc.* 32: 1680–1683.

(104) STIEGER, A. 1913 Über das Vorkommen von Hemicellulosen in Wurzelstöcken, Rhizomen und Wurzelknollen. *Ztschr. Physiol. Chem.* 86: 270–282.

(105) STOCKS, H. B. 1917 Colloid chemistry of starch, gums, hemicelluloses, albumin, casein, gluten and gelatin. *First Rt. on Colloid. Chem. and its Ind. App.*, pp. 53–66.

(106) STORER, F. H. 1903 Notes on the occurrence of mannan in the wood of certain trees and in the various roots and fruits. *Bul. Bussey Inst.* 3: 47–68.

(107) SWARTZ, M. D. 1911 Nutrition investigation on the carbohydrates of lichens, algae and related substances. *Trans. Conn. Acad. of Arts and Sci.* 16: 247–382.

(108) TELLER, G. L. 1912 The carbohydrates of wheat products and changes in the same during development of the grain. *Orig. Commun. 8th Internatl. Cong. Appl. Chem.* 13: 273–278.

(109) TENNEY, F. G., AND WAKSMAN, S. A. 1929 Composition of natural organic materials and their decomposition in the soil. IV. The nature and rapidity of decomposition of the various organic complexes in different plant materials, under aerobic conditions. *Soil Sci.* 28: 55–84.

(110) TESTONI, G. 1923 Methylpentosans in cereals. *Staz. Sper. Agrar. Ital.* 56: 378–385. (*Chem. Abs.* 18: 1345.)

(111) TOLLENS, B. 1888 Kurzes Handbuch der Kohlenhydrate. Breslau.

(112) TOLLENS, B. 1896 Über die in den Pflanzenstoffen und besonders den Futtermitteln enthaltenen Pentosane, ihre Bestimmungsmethoden und Eigenschaften. *Jour. Landw.* 44: 171–194.

(113) TOTTINGHAM, W. E., ROBERTS, R. H., AND LEPKOVSKY, S. 1921 Hemicellulose of apple wood. *Jour. Biol. Chem.* 45: 407–414.

(114) TSUKAMOTO, M. The formation of mannan in *Amorphophallus Konjak*. *Imp. Univ. Col. Agr. (Tokyo) Bul.* 2: 406–408.

(115) VAN HAARST, J., AND OLIVIER, S. C. J. 1914 Determination of pentosans. *Chem. Weekblad.* 11: 918–925.

(116) VAN LAER, M. H., AND MASSCHELEIN, A. 1923 L'évolution des pentosanes de l'orge au cours de la germination. *Bul. Soc. Chim. Belg.* 32: 402–404.

(117) VERHULST, J. H., PETERSON, W. H., AND FRED, E. B. 1923 Distribution of pentosans in the corn plant at various stages of growth. *Jour. Agr. Res.* 23: 655–665.

(118) WAKSMAN, S. A. 1929 Chemical and microbiological principles underlying the decomposition of green manures in the soil. *Jour. Amer. Soc. Agron.* 21: 1–18.

(119) WAKSMAN, S. A. 1930 Chemical composition of peat and the rôle of microorganisms in its formation. *Amer. Jour. Sci.* 19: 32–54.

(120) WAKSMAN, S. A., TENNEY, F. G., AND DIEHM, R. A. 1929 Chemical and microbiological principles underlying the transformation of organic matter in the preparation of artificial manures. *Jour. Amer. Soc. Agron.* 21: 533–546.

(121) WAKSMAN, S. A., AND DIEHM, R. A. 1929 Chemical and microbiological principles underlying the transformation of organic matter in stable manure in the soil. *Jour. Amer. Soc. Agron.* 21: 795–809.

(122) WAKSMAN, S. A., AND STEVENS, K. R. 1929 Processes involved in the decomposition of wood with reference to the chemical composition of fossilized wood. *Jour. Amer. Chem. Soc.* 51: 1187–1196.

(123) WAKSMAN, S. A., AND TENNEY, F. G. 1927 Composition of natural organic materials and their decomposition in the soil: I. Methods of quantitative analysis of plant materials: II. Influence of age of plant upon the rapidity and nature of its decomposition-rye plants. *Soil Sci.* 24: 275–283, 317–334.

(124) WAKSMAN, S. A., AND STEVENS, K. R. 1928 Contribution to the chemical composition of peat: I. Chemical nature of organic complexes in peat and methods of analysis: II. Chemical composition of various peat profiles. *Soil Sci.* 26: 113–137, 239–252.

(125) WAKSMAN, S. A., TENNEY, F. G., AND STEVENS, K. R. 1928 The rôle of microorganisms in the transformation of organic matter in forest soils. *Ecology* 9: 126–144.

(126) WICHERS, J. L., AND TOLLENS, B. 1910 Über die Pentosane einiger Holzpilze. *Jour. Landw.* 58: 238–242.

(127) WINTERSTEIN, E. 1893 Ueber ein im Steinpilz (*Boletus edulis*) enhalten Kohlenhydrat. *Ber. Deut. Bot. Gesell.* 26: 3098–3099.

(128) WINTERSTEIN, E. 1895, 1897 Zur Kenntnis der in den Membranen der Pilze enthaltenen Bestandtheile. *Ztschr. Physiol. Chem.* 19: 521–562 (1895); 21: 152–168 (1897).

(129) WISE, L. F. 1930 The chemistry of the polysaccharides with special reference to cellulose and the hemicelluloses. *Cellulose* 1: 68–76.

ON THE DECOMPOSITION OF HEMICELLULOSES BY MICRO-ORGANISMS: II. DECOMPOSITION OF HEMICELLULOSES BY FUNGI AND ACTINOMYCES[1]

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It has been variously shown that a large number of fungi are capable of decomposing different hemicelluloses, using these carbohydrates as sources of energy and for the building up of microbial cell substance. The chemical processes involved in the decomposition of the various hemicelluloses, especially the nature of the products formed, are still little known. Certain species of *Aspergillus*, for example, have been shown (1) to be able to produce enzymes hydrolyzing mannans and galactans, when grown in solutions containing the respective sugars. Representatives of the genera *Mucor*, *Rhizopus*, *Botrytis*, *Cladosporium*, *Thamnidium*, *Penicillium*, *Trichothecium*, etc. were found by Schellenberg (7) capable of attacking various hemicelluloses. A very specific difference exists, however, in the ability of the different microörganisms to decompose cellulose and hemicelluloses. The Mucorales, for example, are unable as a group to attack pure cellulose but are able to utilize very readily a number of hemicelluloses. Hemicelluloses of different origin differ markedly in this connection, due probably to a difference in chemical nature. The hemicellulose of the endosperm of dates, which gives on hydrolysis with acids galactose and mannose, was found to be very resistant to decomposition by microörganisms; out of 16 species of fungi tested, only 2 (*Penicillium* sp. and *Trichothecium roseum*) were able to attack this carbohydrate. On the other hand, the hemicellulose of *Lupinus hirsutus*, which gives on hydrolysis with dilute acids galactose and arabinose, was readily decomposed by a number of fungi, including species of *Mucor*, *Rhizopus*, *Botrytis*, and *Cladosporium*. According to Otto (4), species of *Mycogone*, *Stemphylium*, *Starchybotrys*, and various other fungi are also capable of attacking the hemicellulose of the date endosperm.

Fungi can attack readily xylans and other pentosans (6, 9). The majority of investigations dealing with the decomposition of pentosans by microorganisms were usually carried out by inoculating a nutrient medium containing a certain plant material with a specific organism, incubating for a certain period of time and measuring the rapidity of the disappearance of the pen-

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tosan by the production of furfural on distillation with 12 per cent hydrochloric acid. Because of the fact, however, that furfural is produced under these conditions both from pentosans and from uronic acid complexes, one can readily recognize that this method of measurement gives only a very imperfect idea of the nature of the substance that has undergone decomposition. It was found (8), for example, that the source of the pentosan has an influence upon the rapidity of its decomposition by specific organisms. About 50 per cent of the pentosan in corn forage was destroyed by pure cultures of fungi in 100 days, and only 35 per cent of the pentosan in the rye straw in 300 days. This difference may be due not so much to the accompanying complexes in the different plant materials, but largely to the different chemical nature of the hemicelluloses in these materials. *Asp. fumigatus* was most active in decomposing the pentosan and *Rhizopus nigricans* least; the former is known to be a cellulose-decomposing organism, whereas the latter cannot attack cellulose. The ability of certain specific microörganisms to bring about the decomposition of specific hemicelluloses, such as pectins, has an important application in certain plant diseases (2), in the preparation of pectin from fruits (5), in the processes of retting of flax, and in the decomposition of plant and microbial residues in composts and in soil.

In their relation to hemicelluloses, microörganisms possess a definite specificity, more so than in the case of the microbes attacking cellulose and proteins, due both to the specific physiology of the different organisms and to the specific chemical nature of the different hemicelluloses. Some microörganisms will attack hemicelluloses more readily during the early stages of decomposition of a plant material, and later attack the cellulose; however, some hemicelluloses are more resistant to the action of microörganisms than is cellulose (11). Certain species of fungi, like yeasts, are entirely unable to attack hemicelluloses (3, 10).

The total number of fungi capable of decomposing hemicelluloses is considerably greater than the number attacking pure cellulose. This ability is not limited to any one group, but is the property of numerous organisms belonging to the Phycomycetes, Ascomycetes, Fungi Imperfecti, and Basidiomycetes. The information concerning the ability of these various fungi to decompose specific hemicelluloses is still very limited. Still less is known of the behavior of actinomyces in this respect.

In the following investigations, an attempt has been made to study the decomposition of certain purified hemicellulose preparations, which have been freed from sugars, cellulose, and lignin. The methods of obtaining these "hemicelluloses" have been described previously (12). The fungi used for these studies were selected from the laboratory stock cultures. The actinomyces, however, were freshly isolated from a fertile soil in which the specific hemicelluloses were undergoing active decomposition. No attempt has been made to identify and describe the various species of actinomyces thus isolated, since they were considered in these experiments more as types representing

this particular genus in its ability to decompose hemicelluloses; only active organisms have been selected for this purpose.

DECOMPOSITION OF MANNAN

The decomposition of mannan, as well as that of the other hemicellulose preparations, was studied both in liquid and in sand culture media. In making up these media, the following nutrients were added to 100-cc. portions of solution, or to 100-gm. portions of sand containing 20 cc. water:

	gm.
$(NH_4)_2HPO_4$	0.050
K_2HPO_4	0.050
$MgSO_4$	0.010
NaCl	0.010
$CaCl_2$	0.010
$FeSO_4$	0.001
Mannan	0.500

These portions of liquid or sand media containing the nutrients were placed in a series of flasks, sterilized, inoculated with the various pure cultures of the organisms, and incubated at 27 to 28°C. for a period of 42 days.

At the end of the incubation period the contents of the flasks were analyzed for total hemicellulose and for ammonia nitrogen. Instead of the hemicellulose being boiled with acid under a reflux condenser for 5 hours, the hydrolysis was effected by autoclaving with 2 per cent HCl under 15 pounds pressure for 30 minutes. This method of hydrolysis can be employed only when there are no other polysaccharides present which would give reducing sugars upon hydrolysis with dilute acids under pressure. Ammonia determinations were made by distilling an aliquot portion of the culture with magnesium oxide.

The decomposition of the mannan in the sand medium by pure cultures of fungi is shown in table 1. All of the fungi tested were found to be able to decompose the mannan very actively. The maximum decomposition was noted in the case of the *Rhizopus*, which brought about the destruction of 95 per cent of the mannan. The *Penicillium* was found to be the least active of the fungi tested, decomposing in this experiment only 76 per cent of the mannan present in the medium. When one compares the amount of nitrogen required by the fungi to bring about the decomposition of the mannan, one finds that, on an average, 35.5 parts of mannan were decomposed per one part of inorganic nitrogen assimilated. In addition to bringing about the greatest decomposition of the mannan, the *Rhizopus* was also the organism that required the least nitrogen, 49.2 parts of the mannan being decomposed per one part of nitrogen assimilated. It is of considerable interest and possibly of practical importance to call attention to the fact that the three representative genera of Phycomycetes, namely *Zygorhynchus*, *Cunninghamella*, and *Rhizopus*, which are known (13) to be unable to decompose true cellulose, proved to be most active in the decomposition of the hemicellulose mannan. These three

organisms were not only as active in decomposing the mannan as the true cellulose decomposing fungi, namely *Trichoderma*, *Humicola*, and *Asp. fumigatus*, but they required less nitrogen for this decomposition than the other organisms, at least in sand media.

TABLE 1

The decomposition of mannan in sand medium by pure cultures of fungi

ORGANISM	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control medium	305.5	.....	9.6	...	....
Zygorhynchus	25.9	279.6	2.9	6.7	41.7
Cunninghamella	30.6	274.9	2.3	7.3	37.7
Rhizopus	15.1	290.4	3.7	5.9	49.2
Penicillium	71.8	233.7	2.4	7.2	32.5
Trichoderma	20.5	285.0	0.8	8.8	32.4
Humicola	55.6	249.9	1.8	7.8	32.0
Asp. niger	59.9	245.6	1.9	7.7	31.9
Asp. fumigatus	28.4	277.1	1.8	7.8	35.5

TABLE 2

Decomposition of mannan in solution medium by pure cultures of fungi

ORGANISM	pH	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	5.4	382.5	.....	9.6	...	....
Zygorhynchus	5.0	255.2	127.3	6.0	3.6	32.6
Cunninghamella	5.2	21.6	360.9	1.0	8.6	41.9
Rhizopus	4.8	14.9	367.6	0.6	9.0	40.8
Penicillium	5.2	8.1	374.4	2.8	6.8	55.1
Trichoderma	5.2	270.5	112.0	5.2	4.4	25.4
Humicola	5.0	21.6	360.9	1.2	8.4	42.8
Asp. niger	5.0	21.6	360.9	0.6	9.0	40.1
Asp. fumigatus	5.2	17.1	365.4	0.8	8.8	40.3

In order to compare the influence of the nature of the medium upon the rapidity and nature of decomposition of mannan by various fungi, the same organisms were inoculated into a sterile solution containing mannan and inorganic nutrient salts. The results obtained in this experiment are reported in table 2. Colorimetric determinations of the hydrogen-ion concentration of the medium as expressed by their pH value, were also made in all the cul-

tures. The increase in the H-ion concentration in the inoculated flasks indicates the formation of organic acids during the decomposition process. The results are not in exact conformity with those obtained in the sand medium. *Penicillium*, for example, which decomposed the least amount of mannan in the sand medium, brought about the greatest decomposition in the solution me-

TABLE 3

The decomposition of mannan in sand medium by pure cultures of soil actinomyces

ORGANISM	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	305.5	.....	9.6	...	....
Actinomyces 26	31.3	274.2	1.2	8.4	32.6
Actinomyces 40	15.1	290.4	4.1	5.5	52.8
Actinomyces 48	25.9	279.6	5.8	3.8	73.6
Actinomyces 50	17.8	287.7	3.7	5.9	48.8
Actinomyces 54	26.1	279.4	4.3	5.3	52.7
Actinomyces 33	23.9	281.6	4.7	4.9	57.5
Actinomyces 51	44.1	261.4	3.2	6.4	40.8
Actinomyces 76	32.9	272.6	2.5	7.1	38.4

TABLE 4

Decomposition of mannan in solution medium by pure cultures of soil actinomyces

ORGANISM	pH	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	5.4	382.5	.....	...	...	....
Actinomyces 26	5.2	203.9	178.6	4.4	5.2	34.4
Actinomyces 40	5.2	28.4	354.1	2.8	6.8	52.1
Actinomyces 48	5.2	127.8	254.7	2.2	7.4	34.4
Actinomyces 50	5.2	194.0	188.5	3.4	6.2	30.4
Actinomyces 54	5.2	208.8	173.7	5.8	3.8	45.7
Actinomyces 33	5.0	164.3	218.2	1.2	8.4	26.0
Actinomyces 51	5.2	99.0	283.5	0.2	9.4	30.2
Actinomyces 76	5.2	176.9	205.6	1.6	8.0	25.7

dium, 97.9 per cent of the mannan originally present having disappeared during the period of decomposition. The ratio between the decomposition of the hemicellulose and the nitrogen utilization was wider in the solution cultures than in the sand cultures.

The following two experiments deal with the decomposition of the mannan by a series of actinomyces. The action of these organisms upon the hemi-

cellulose in a sand medium is reported in table 3. The process of decomposition was carried out rapidly by the actinomyces as well, although these organisms are usually reputed to be slow growing; *Actinomyces 40* brought about the greatest decomposition, 95 per cent of the mannan having disappeared in the given period of time. The lowest amount of decomposition was noted in the case of *Actinomyces 51*, but even in this case 86 per cent of the mannan originally present in the medium has disappeared. The utilization of nitrogen in the decomposition of this polysaccharide by actinomyces shows more variation than in the case of the fungi: the average ratio of mannan decomposed to the inorganic nitrogen assimilated was 47.2 with 73.6 as the maximum and 32.6 as the minimum, this ratio being wider than in the case of the fungi.

The results of the decomposition of mannan by pure cultures of actinomyces in a liquid medium are given in table 4. The changes in pH in these cultures were not very great. Only in one case, namely with the *Actinomyces 33*, was

TABLE 5

The decomposition of mannan in salep root by pure cultures of fungi and actinomyces

ORGANISM	pH	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	7.0	660.4	.....	62.0	....	....
Zygorhynchus	6.4	224.6	435.8	57.6	4.4	99.0
Rhizopus	6.8	47.5	612.9	48.4	13.6	45.0
Penicillium	7.0	39.4	621.0	46.4	15.6	39.8
Trichoderma	6.6	340.7	319.7	56.4	5.6	57.1
Humicola	7.0	83.2	577.4	54.0	8.0	72.2
Actinomyces	6.4	44.8	615.6	48.0	14.0	44.0

the pH value lowered by 0.4. The average amount of mannan decomposed by actinomyces in solution culture was 67 per cent of the total. However, the *Actinomyces 40* decomposed 92 per cent of the total mannan present in the medium. On the average, one part of inorganic nitrogen was assimilated by the actinomyces for every 34.8 parts of mannan destroyed in liquid medium. These organisms decomposed considerably larger amounts of hemicellulose per unit of inorganic nitrogen assimilated in sand than in solution media, or a larger quantity of nitrogen is transformed from an inorganic into an organic form by the same organism when it grows in a liquid medium than in a fully aerated sand medium.

The results presented here prove beyond any doubt that when mannans are applied to soil in the complex plant materials, they soon disappear, if conditions for their decomposition are favorable. The above investigations were carried out by the use of purified mannan. In order to determine whether

or not the extraction and preparation of the mannan had any influence upon the nature and rapidity of its decomposition, the utilization of mannan in its natural state, namely in the form of salep root by microörganisms, was studied. These studies were carried out in liquid medium only. The results, reported in table 5, show that from 48.4 to 94.0 per cent of the mannan in the natural product was decomposed by the fungi; in other words, the decomposition is as rapid as that of the purified preparation. The actinomyces decomposed 93.2 per cent of the mannan added to the medium. The nitrogen consumption by the organisms bringing about the decomposition of the mannan in the salep root is considerably less than in the case of the purified mannan preparation, 62.6 units of the mannan being destroyed per unit of nitrogen assimilated, in the case of the fungi, and 44 parts in the case of the actinomyces. The nitrogen utilization value in this experiment should, of course, not be considered as

TABLE 6

The decomposition of xylan in sand medium by pure cultures of fungi

ORGANISM	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	152.0	....	8.6	...	....
Zygorhynchus	107.1	44.9	6.1	2.5	18.0
Cunninghamella	67.0	85.0	3.4	5.2	16.3
Rhizopus	79.2	72.8	...	...	....
Penicillium	79.2	72.8	3.9	4.7	15.2
Trichoderma	65.7	86.3	2.8	5.8	14.9
Humicola	88.2	63.8	3.4	5.2	12.3
Asp. fumigatus	52.9	99.1	2.8	5.6	22.5

exact as in the experiments with the purified mannan, because in the former instance the organisms had at their disposal some of the nitrogen present in the salep root itself, which they used in preference to the added inorganic nitrogen.

DECOMPOSITION OF XYLAN BY MICROÖRGANISMS

The experiments dealing with the decomposition of the xylan preparation were carried out in the same manner as outlined for the decomposition of the mannan. The flasks were inoculated with organisms which had shown, in previous experiments (not reported here), that they were capable of decomposing xylan. The incubation period was 6 weeks at a temperature of 27 to 28°C. The methods of quantitative analysis were also the same as those employed in the previous experiments.

All the fungi tested (table 6) decomposed the xylan, but to a varying extent.

The range of decomposition was 29.5 and 83.0 per cent of the xylan in the sand medium. *Aspergillus fumigatus* brought about the maximum destruction of the xylan, whereas *Zygorhynchus* attacked this carbohydrate to the least extent. In bringing about this process, the fungi consumed a large amount of nitrogen, the average being about one unit of nitrogen consumed for every 16.7 parts of xylan decomposed. The reason for this large consumption of

TABLE 7

The decomposition of xylan in solution medium by pure cultures of fungi

ORGANISM	pH	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm*	*mgm.*	*mgm.*	
Control	6.2	287.3	.....	8.6	...	....
Zygorhynchus	6.2	235.8	51.5	...	...	....
Cunninghamella	6.0	132.6	154.7	3.2	5.4	28.7
Rhizopus	6.2	232.2	55.1	6.6	2.0	27.6
Penicillium	6.2	129.0	158.3	5.0	3.0	52.8
Trichoderma	6.2	126.0	161.3	2.6	6.0	26.9
Humicola	6.2	92.4	184.9	3.4	5.2	35.6
Asp. niger	5.8	129.0	158.3	1.6	7.0	22.6
Asp. fumigatus	5.8	110.4	176.9	1.2	7.4	23.9

TABLE 8

The decomposition of xylan in sand medium by pure cultures of actinomyces

ORGANISM	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	152.0	.....	8.6	...	....
Actinomyces 26	38.7	133.3	2.3	6.3	18.0
Actinomyces 40	25.2	126.8	3.3	5.3	23.9
Actinomyces 50	29.7	122.3	2.2	6.4	19.1

nitrogen with xylan as a source of energy in the sand medium remains to be determined.

The decomposition of the xylan in solution medium by pure cultures of fungi is shown in table 7. The greatest decomposition was brought about by *Humicola* and *Asp. fumigatus*, which destroyed 64 and 62 per cent of the xylan respectively in 6 weeks. The *Rhizopus* and *Zygorhynchus* brought about the least decomposition, so that the average amount of xylan destroyed by the various fungi was 49.2 per cent. In the case of the liquid medium 27.6 parts of xylan were decomposed for every unit of nitrogen assimilated.

The decomposition of xylan by actinomyces is reported in tables 8 and 9. The results of decomposition in the sand medium, where only three strains were employed, show that these organisms are capable of decomposing xylans very rapidly: on the average, 79.5 per cent of the xylan present in the medium disappeared in 6 weeks. The fact that 20.3 parts of the xylan were decomposed

TABLE 9

The decomposition of xylan in solution medium by pure cultures of actinomyces

ORGANISM	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	287.3	.....	8.6	...	....
Actinomyces 26	178.9	108.4	5.2	3.4	31.9
Actinomyces 40	186.6	100.7	...	...	....
Actinomyces 48	186.6	100.7	4.4	4.2	24.0
Actinomyces 50	235.8	51.5	7.8	0.8	64.4

TABLE 10

The decomposition of xylan in corn cobs in solution medium by pure cultures of fungi and actinomyces

ORGANISM	pH	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	6.8	405.5	.....	30.0	...	....
Rhizopus	6.6	187.9	237.6	....	...	....
Penicillium	6.6	223.6	181.9	23.6	6.4	28.4
Trichoderma	6.6	331.0	74.5	28.0	2.0	37.3
Humicola	6.5	299.7	105.8	25.6	4.4	24.1
Cunninghamella	6.6	191.2	214.3	28.4	1.6	....
Asp. niger	6.5	302.9	102.6	28.0	2.0	51.3
Asp. fumigatus	6.5	187.5	218.0	25.2	4.8	45.4
Actinomyces 26	6.6	372.1	33.4	28.4	1.6	20.9
Actinomyces 50	6.5	362.9	42.6	27.6	2.4	17.8
Actinomyces 40	6.5	306.2	98.3	....	...	....

for every unit of nitrogen assimilated shows an extensive energy consumption just as in the case of the fungi. In liquid medium (table 9) the actinomyces proved to be much less efficient, because of unfavorable cultural conditions. On the average, 31.4 per cent of the xylan originally present in the medium was decomposed; one part of nitrogen was assimilated for every 40.1 parts of xylan destroyed.

In order to study the decomposition of the xylan in the natural state, ground corn cobs were employed. Xylan makes up about 30 per cent of the total constituents of the dry corn cobs. Although the microörganisms were capable of attacking the other constituents in the corn cobs as well, only the transformation of the xylan portion was taken into consideration. The studies were carried out in a manner similar to the previous experiments, using a solution medium. The results are given in table 10. On the average, the fungi decomposed 43.1 per cent of the xylan originally present in the corn cobs, whereas the actinomyces brought about the disappearance of only 14.3 per cent of the xylan. These results show definitely that a certain chemical complex may be decomposed by microörganisms in a different manner in a purified condition than when present in the natural state in the plant material. Whether this is due to the influence of the other organic complexes in the plant upon the

TABLE 11

The decomposition of galactan in sand medium by pure cultures of fungi

ORGANISM	GALACTAN		AMMONIA-NITROGEN		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	170.2	....	9.3	...	....
Zygorhynchus	127.8	42.4	7.9	1.4	30.3
Cunninghamella	127.8	42.4	8.7	0.6	70.7
Rhizopus	142.2	28.0	7.6	1.7	16.5
Penicillium	122.4	47.8	8.7	0.6	79.6
Trichoderma 27	108.9	61.3	7.7	1.6	38.3
Humicola	86.4	83.8	...	...	....
Asp. niger	99.0	71.2	7.6	1.7	41.9
Asp. fumigatus	108.9	61.3	7.9	1.4	43.8

decomposition of the specific complex, or due to its chemical or possibly physical modification in the process of preparation and purification remains to be determined. The limitations of nitrogen utilization studies when impure preparations are employed being considered, the fungi brought about the decomposition of 34.5 parts of xylan per unit of nitrogen assimilated, and the actinomyces, 19.4 parts.

DECOMPOSITION OF GALACTAN BY MICROÖRGANISMS

The decomposition of galactan by various microörganisms was carried out in a manner similar to the studies of mannan and xylan decomposition. The galactan was found to be considerably more resistant to attack by microorganisms than the other two carbohydrates. There was also observed considerably greater variation among the various organisms in their ability to decompose the galactan. The decomposition of galactan in the sand medium

by pure cultures of fungi is reported in table 11. The results show that, on the average, 33.3 per cent of the galactan disappeared as a result of the activities of the fungi. The amount of galactan decomposed per unit of nitrogen assimilated is rather variable, the average value obtained being about 35.

The decomposition of galactan in solution media by pure cultures of fungi (table 12) is quite similar to that in sand media. The nitrogen utilization in this experiment could not be computed with any degree of accuracy because

TABLE 12

The decomposition of galactan in solution medium by pure cultures of fungi

ORGANISM	pH	GALACTAN		AMMONIA-NITROGEN	
		Found	Decomposed	Found	Assimilated
		mgm.	*mgm.*	*mgm.*	*mgm.*
Control	7.2	306.9	.....	9.3	...
Zygorhynchus	7.6	218.7	88.2	9.0	0.3
Rhizopus	7.4	202.5	104.4	9.0	0.3
Penicillium	7.4	202.5	104.4	8.7	0.6
Cunninghamella	7.6	261.9	45.0	9.3	...
Trichoderma	7.4	192.6	114.3	8.7	0.6
Humicola	7.5	208.8	98.1	9.3	...
Asp. niger	7.4	192.6	114.3	8.7	0.6
Asp. fumigatus	7.2	192.6	114.3	9.0	0.3

TABLE 13

The decomposition of galactan in sand medium by pure cultures of soil actinomyces

ORGANISM	GALACTAN		AMMONIA-NITROGEN		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	170.2	.....	9.3	...	....
Actinomyces 26	119.7	50.5	8.1	1.2	42.0
Actinomyces 33	99.0	71.2	8.3	1.0	71.2
Actinomyces 35	135.9	34.3	6.9	2.4	14.7
Actinomyces 40	97.2	73.0	7.4	1.9	38.4
Actinomyces 50	69.3	100.9	7.8	1.5	67.3

of the inconsistent results obtained. In view of the fact that the galactan preparation contained a certain amount of combined nitrogen, which some of the organisms at least could utilize, the exact rôle played by the inorganic nitrogen under these conditions in favoring decomposition could of course not be ascertained. There was an average decomposition of about 30 per cent of the galactan present in the medium.

The destruction of the galactan by actinomyces in sand culture is given in table 13. It has been observed in preliminary experiments that the galactan

supports an active flora of actinomyces in the soil, and one might, therefore, expect that this group of soil microörganisms would decompose a considerable part of the galactan. The actinomyces employed in this experiment actually brought about the disappearance of about 38.8 per cent of the galactan originally present in the medium. These organisms were able to decompose, on

TABLE 14

The decomposition of galactan in solution medium by pure cultures of soil actinomyces

ORGANISM	pH	GALACTAN		AMMONIA-NITROGEN	
		Found	Decomposed	Found	Assimilated
		mgm.	*mgm.*	*mgm.*	*mgm.*
Control	7.2	306.9	.....	9.3	...
Actinomyces 26	7.5	218.7	88.2	9.7	...
Actinomyces 33	7.4	202.5	104.2	9.7	...
Actinomyces 35	7.3	258.3	48.6	9.7	...
Actinomyces 40	7.4	216.9	90.0	9.0	0.3
Actinomyces 50	6.6	286.2	20.7	8.3	1.0

TABLE 15

The decomposition of galactan in Irish moss in solution by pure cultures of microörganisms

ORGANISM	GALACTAN		AMMONIA-NITROGEN		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilpted	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	382.3	.....	50.0	...	....
Zygorhynchus	265.7	116.6	43.2	6.8	17.1
Rhizopus	259.7	122.6	46.0	4.0	30.7
Penicillium	263.0	119.3	43.2	6.8	17.6
Cunninghamella	244.6	137.7	43.2	6.8	20.3
Trichoderma	265.7	116.6	43.6	6.4	18.2
Humicola	271.6	110.7	46.8	3.2	34.6
Asp. niger 7	281.3	101.0	44.0	6.0	16.8
Asp. fumigatus	271.6	110.7	48.4	1.6	69.2
Actinomyces 26	263.0	119.3	47.6	2.4	49.7
Actinomyces 33	268.9	113.4	48.8	1.2	94.5
Actinomyces 35	287.3	95.0	48.0	2.0	47.5
Actinomyces 40	253.8	128.5	46.8	3.2	40.2
Actinomyces 50	241.9	140.4	44.8	5.2	27.0

the average, 46.7 parts of galactan for every unit of nitrogen assimilated in the synthesis of their protoplasm. Here again, it is difficult to tell how much the utilization of the organic nitrogen in the galactan preparation modified the consumption of inorganic nitrogen for the synthesis of the microbial cell substance.

The decomposition of galactan in the solution medium by actinomyces is shown in table 14. The results are very variable, similar to those noted in the decomposition of the galactan by the fungi. About 22.9 per cent of the total galactan in the medium was destroyed by the actinomyces. The utilization by the organisms, under these conditions, of the organic nitrogenous constituents of the galactan preparation is indicated by the fact that in certain cultures there was more ammonia at the end of the experiment than at the beginning. The actinomyces are known to be organisms capable of attacking

TABLE 16

Evolution of carbon dioxide in the decomposition of mannan by pure cultures of fungi

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION						
	2	4	6	11	18	28	42
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control	0.1	0.3	0.7	2.0	3.5	4.0	5.2
Zygorhynchus	15.1	29.3	39.7	49.9	55.7	62.1	72.0
Rhizopus	21.0	30.6	51.8	60.2	63.3	66.0	69.1
Penicillium	9.4	27.0	50.4	60.2	63.9	67.4	70.3
Trichoderma	15.4	34.0	44.2	50.1	58.4	66.5	71.0
Humicola	17.4	37.1	55.7	61.2	64.8	67.0	70.2

TABLE 17

Evolution of carbon dioxide in the decomposition of mannan in sand medium by pure cultures of soil actinomyces

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION						
	2	4	6	11	18	28	42
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control	0.1	0.3	0.7	2.0	3.5	4.0	5.2
Actinomyces 26	11.3	17.5	29.9	43.4	55.9	71.5	91.3
Actinomyces 40	15.1	31.8	47.1	62.2	73.6	80.4	85.8
Actinomyces 48	5.9	13.2	35.5	72.1	84.4	89.6	95.9
Actinomyces 50	11.3	30.2	43.3	64.9	79.8	86.0	89.9

organic nitrogenous complexes as sources of energy in preference even to readily available carbohydrates.

The decomposition of the galactan in the unmodified plant material, namely the Irish moss, was also studied. It is to be remembered of course that this plant material contained other organic complexes in addition to the galactan, which were undergoing decomposition. This would influence particularly the nitrogen consumption by the microörganisms. However, only the decomposition of the galactans was considered in this experiment. The results

presented in table 15 bring out again the resistance of the galactan in the natural state towards decomposition by various microörganisms. The fungi

TABLE 18

Evolution of carbon dioxide in the decomposition of mannan in salep root by microörganisms

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION						
	8	11	13	15	18	24	30
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control	1.0	1.5	2.1	2.8	3.5	4.0	4.5
Zygorhynchus	14.7	25.4	30.8	34.1	37.7	44.0	48.6
Rhizopus	32.5	59.3	76.1	88.9	97.5	109.9	117.7
Penicillium	14.3	37.1	52.3	63.4	76.8	93.6	104.1
Trichoderma	12.4	33.8	51.3	61.1	66.8	75.3	78.3
Actinomyces 26	5.8	23.3	44.8	61.0	74.2	95.7	102.7

TABLE 19

Evolution of carbon dioxide from xylan by pure cultures of fungi

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION				
	4	6	18	28	42
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control	0.3	0.8	2.5	3.8	5.1
Zygorhynchus	2.0	2.4	4.1	6.0	10.1
Rhizopus	2.8	3.9	7.0	10.3	13.5
Penicillium	9.7	24.7	39.4	42.1	46.4
Trichoderma	14.4	29.5	43.4	46.7	48.7
Humicola	19.2	30.4	39.6	42.3	47.3

TABLE 20

Evolution of carbon dioxide from xylan by pure cultures of actinomyces

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION				
	4	6	18	28	42
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control	0.3	0.8	2.5	3.8	5.1
Actinomyces 26	12.8	25.6	48.1	56.8	65.6
Actinomyces 40	17.7	32.4	52.3	60.8	67.8
Actinomyces 50	0.4	2.1	18.5	42.0	52.4

decomposed only 31.0 per cent of the hemicellulose in a period of 6 weeks and the actinomyces 31.2 per cent. It is interesting to note two phenomena brought out in this experiment; namely, the fact that the active cellulose-

decomposing fungi *Trichoderma* and *Asp. fumigatus* decomposed no more galactan, in fact even less, than the non-cellulose decomposing Phycomycetes; the abundant decomposition of galactan by actinomyces confirms the previous observations concerning the use of purified preparations. The fungi and actinomyces assimilated one part of nitrogen for every 27.9 and 51.8 parts of galactan decomposed respectively. This difference in ratio is due both to the greater nitrogen consumption by the fungi and to greater ability to utilize the organic nitrogen in the moss by the actinomyces.

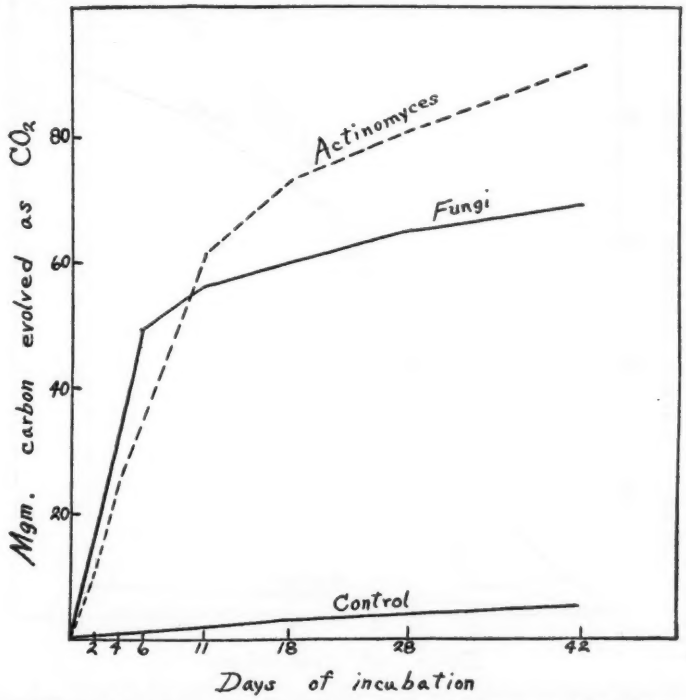

FIG. 1. THE RATE OF DECOMPOSITION OF MANNAN IN SAND MEDIUM BY PURE CULTURES OF FUNGI AND ACTINOMYCES

In all the previous experiments only the total quantities of hemicellulose decomposed over a definite period of time were considered. In order to ascertain also the relative speed of decomposition of the various hemicelluloses, the carbon dioxide evolved in the process of decomposition was measured at short intervals over an extended period of time. The sand medium was utilized for this purpose. Carbon dioxide-free air was drawn through the cultures to displace the carbon dioxide in the flasks and this gas was absorbed in $N/6$ barium hydroxide solution. The excess barium hydroxide was titrated back with standard oxalic acid solution, at frequent intervals.

Table 16 shows the accumulative evolution of carbon dioxide, reported as milligrams of carbon, by the various fungi from mannan in sand culture. About 170 mgm. of carbon were added in the form of mannan; a little over one-third of this amount was liberated as CO_2 in 42 days. Since the fungi used represent several distinct types, it would appear that the mannan is readily decomposed by fungi in general and at about the same rate. Decom-

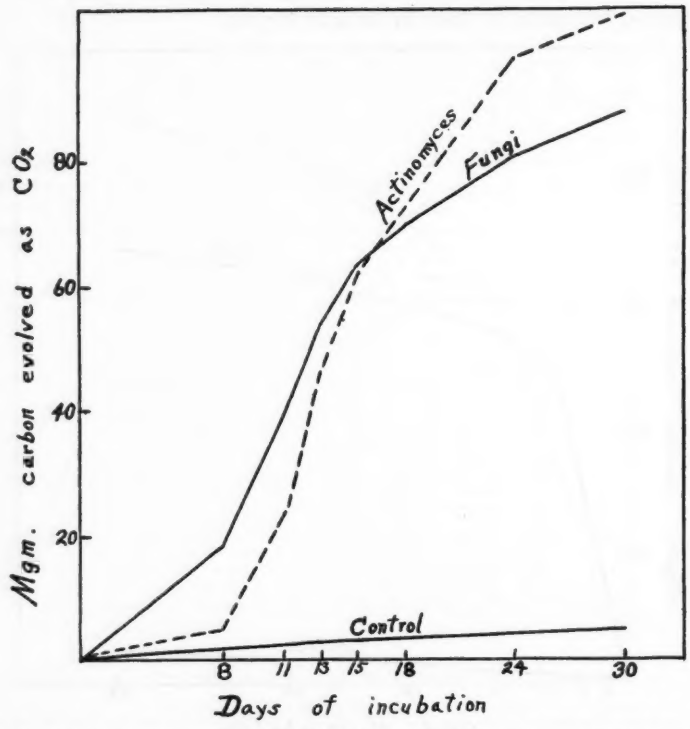

FIG. 2. THE RATE OF DECOMPOSITION OF MANNAN IN SALEP IN SAND MEDIUM BY PURE CULTURES OF FUNGI AND ACTINOMYCES

position was very rapid during the first 11 days; after that period, the rate of decomposition gradually decreased.

The liberation of carbon dioxide from mannan by actinomyces grown in sand medium is shown in table 17. A marked similarity in the total amounts of carbon evolved is again noted. The actinomyces were at first slower in bringing about the decomposition of the mannan than were the fungi, but they became more active later, finally resulting in the liberation of larger amounts of carbon dioxide. All the actinomyces liberated over one-half of the total carbon present originally in the mannan as carbon dioxide. Since the fungi

produce a more extensive mycelium than do the actinomyces, and use, therefore, more carbon for that purpose; the actual amount of mannan decomposed was probably the same, if not more, in the case of the fungi. This was brought out in tables 1–4.

These results show that, although the method of determination of carbon dioxide evolution as an index for measuring the rapidity of decomposition of plant material is very satisfactory, it cannot be used for comparing the activities of different groups of organisms which differ in their metabolism. This

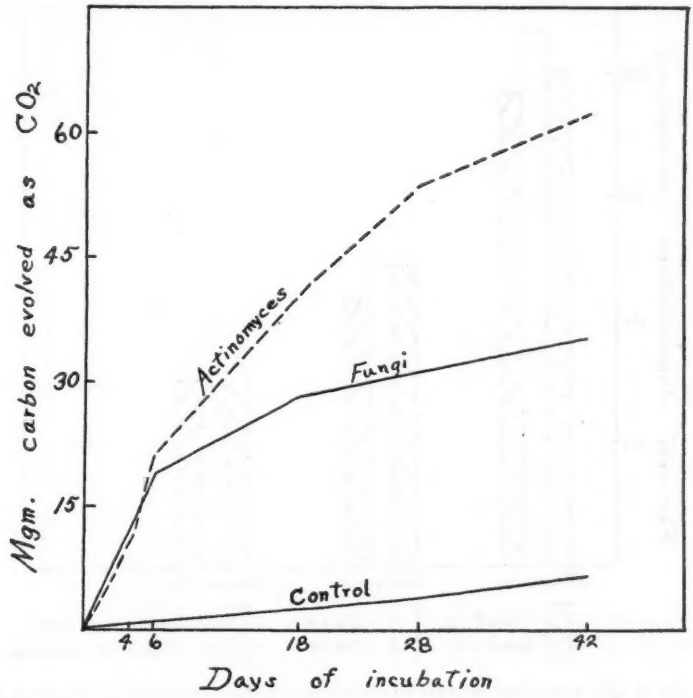

FIG. 3. THE RATE OF DECOMPOSITION OF XYLAN IN SAND MEDIUM BY PURE CULTURES OF FUNGI AND ACTINOMYCES

is true in the study of decomposition of more or less definite chemical complexes, but even more so in the study of unmodified plant materials. The same amount of carbon dioxide given off by different organisms may actually be a result of the decomposition of different plant constituents or different amounts of the same constituent.

Following the same plan of experiment, the evolution of carbon dioxide in the decomposition of natural mannan in the salep root was studied. It is of course possible and even certain that not all the carbon dioxide evolved in

this experiment came from the decomposition of the hemicellulose in the salep, but the mannan content is so large, in proportion to the other constituents, and the microörganisms have shown their ability to decompose the mannan so actively, that one is safe in assuming that most of the carbon liberated came from the mannan. The results of this experiment are given in table 18. The fungi showed the most active decomposition of the mannan in the early stages

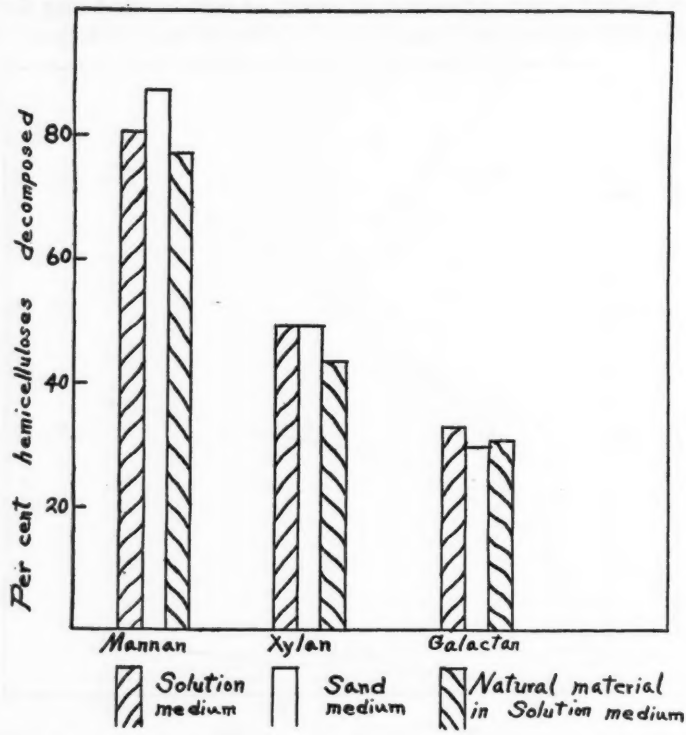

FIG. 4. THE TOTAL DECOMPOSITION OF THE VARIOUS HEMICELLULOSES BY FUNGI IN A PERIOD OF SIX WEEKS

of growth, but they did not slow down as much near the end of the incubation period as they did when the purified mannan was used as a source of energy.

The rate of decomposition of xylan from corn cobs, as shown by the evolution of carbon dioxide, was also studied. The plan of the experiment was identical to that used in the case of the mannan. Table 19 shows the amounts of carbon dioxide, reported as milligrams of carbon, evolved in the decomposition of xylan by pure cultures of fungi in a sand medium. Theoretically, about 207 mgm. of carbon were added to the medium in the form of xylan. The *Zygorhynchus* and *Rhizopus* were quite inactive in this experiment and gave off only

5.0 and 8.4 mgm. of carbon respectively, above the amount found in the uninoculated control. The remaining fungi liberated as carbon dioxide nearly 20 per cent of the carbon originally present in the xylan.

The liberation of carbon dioxide by the actinomyces from xylan in sand medium is given in table 20. Here again, the rapidity of evolution of carbon dioxide by the actinomyces is more gradual than in the case of the fungi, but

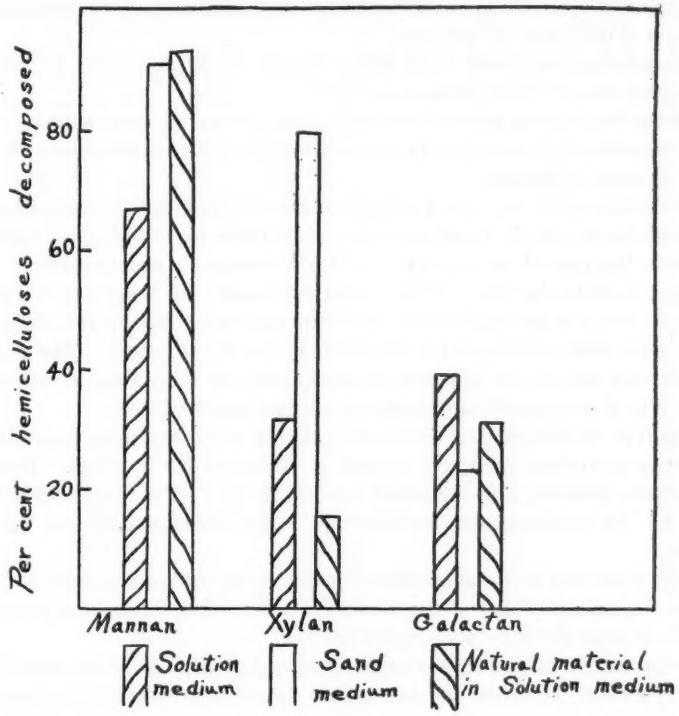

FIG. 5. THE RATE OF DECOMPOSITION OF THE VARIOUS HEMICELLULOSES BY ACTINOMYCES IN A PERIOD OF SIX WEEKS

it is also more prolonged. With one exception, over 25 per cent of the carbon in the xylan was liberated as carbon dioxide by the actinomyces.

Very little can be said at the present time concerning the mechanism of decomposition of the hemicelluloses by fungi and actinomyces. A certain amount of organic acids is formed by some of the fungi. The nature of these acids is not known, the amount varying with the organism, period of incubation, nature and composition of medium, etc. In no case did the actinomyces produce any organic acids in the cultures. This suggests definitely that the different organisms decompose the hemicelluloses in a different manner.

The average rate of decomposition and the total decomposition of the three "hemicelluloses," in a natural and purified state by fungi and actinomyces is brought out graphically in figures 1 to 5.

SUMMARY

A study has been made of the decomposition of several typical hemicelluloses, both in a purified form and in the natural plant material, by certain pure cultures of fungi and actinomyces.

The galactan was found to be more resistant to decomposition by microörganisms than were the mannan and xylan.

All the fungi tested were able to decompose the specific hemicelluloses; the Phycomycetes were as active in this process as the cellulose-decomposing fungi used in these experiments.

The actinomyces were found to be even more active in the decomposition of hemicelluloses than the fungi, especially under favorable conditions of culture.

When the rate of decomposition of hemicellulose by microörganisms was compared, measuring the evolution of carbon dioxide, the fungi were found to be more active in the beginning of the incubation period, but became considerable slower after decomposition has proceeded for about a week. The actinomyces were slow at the beginning of incubation, but maintained a more uniform rate of decomposition throughout a longer period of time.

The type of hemicellulose was found to be the most important determining factor in controlling the actual amount of decomposition by fungi. The actinomyces, however, were influenced not only by the type of hemicellulose but also by the environmental conditions at which decomposition was taking place.

Both fungi and actinomyces liberate considerable amounts of carbon dioxide in the process of destruction of hemicelluloses. The former also produces small amounts of organic acids in this process.

It appears that the process of preparation and purification of the hemicelluloses influences the nature and rate of their decomposition by microörganisms.

REFERENCES

(1) Hérissey, H. 1903 Récherches chimiques et physiologiques sur la digestion des mannanes et des galactanes par la seminase chez les végétaux. Thèse. Paris.

(2) Jones, L. R. 1909 Pectinase, the cytolytic enzyme produced by *Bacillus carotovorus* and certain other soft-rot organisms. N. Y. Agr. Exp. Sta. Tech. Bul. 11.

(3) Lintner, C. J. and Dull. 1891 Über die chemische Nature des Gerstengummi. Chem. Ztg. 15 (Repertorium): 266.

(4) Otto, H. 1916 Untersuchungen über die Auflösung von Zellulosen und Zellwänden durch Pilze. *Beitr. Allg. Bot. 1:* 190-260.

(5) Pitman, G. A., and Cruess, W. V. 1929 Hydrolysis of pectin by various microorganisms. *Jour. Indus. and Engin. Chem.* 21: 1292–1295.

(6) Rege, R. D. 1927 Biochemical decomposition of cellulosic materials, with special reference to the action of fungi. *Ann. Appl. Biol.* 14: 1–44

(7) SCHELLENBERG, H. C. 1908 Untersuchungen über das Verhalten einiger Pilze gegen Hemizellulosen. *Flora* 98: 257–308.

(8) SCHMIDT, E. G., PETERSON, W. H., AND FRED, E. B. 1923 The destruction of pentosans by molds and other microörganisms. *Soil Sci.* 15: 479–488.

(9) SMITH, R. G. 1924 A chemical and pathological study of the xylem of the apple caused by *Polystictus versicolor* Fr. *Phytopathology* 14: 114–118.

(10) TOLLENS, B., AND GLAUBITZ, H. 1897 Über den Pentosan-gehalt verschiedener Materialien, welche zur Ernährung dienen, und in den Gärungs-Industrieen angewendet werden, und über den Verbleib des Pentosans bei den Operationen, welcher die obigen Materialien unterworfen werden. *Jour. Landw.* 45: 97–111.

(11) WAKSMAN, S. A. 1931 Decomposition of the various chemical constituents of fresh plant materials by pure cultures of fungi and bacteria. *Arch. Microb.* 2: 136–154.

(12) WAKSMAN, S. A., AND DIEHM, R. A. 1931 On the decomposition of hemicelluloses by microorganisms. I. Nature, occurrence, preparation and decomposition of hemicelluloses. *Soil Sci.* 32: 73–95.

(13) WAKSMAN, S. A., AND HEUKELEKIAN, H. 1926 Cellulose decomposition by various groups of soil microorganisms. *IV Internatl. Soil Sci. Conf. Rome, Comn.* 3: 1–13.

ON THE DECOMPOSITION OF HEMICELLULOSES BY MICROÖRGANISMS: III. DECOMPOSITION OF VARIOUS HEMICELLULOSES BY AEROBIC AND ANAEROBIC BACTERIA[1]

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Although it has been definitely established that various hemicelluloses are decomposed under both aerobic and anaerobic conditions, the literature on the decomposition of organic complexes by microörganisms contains but few references concerning the ability of specific aerobic and anerobic bacteria to decompose this group of carbohydrates. When compared with the interest aroused by the bacteria decomposing cellulose, the breakdown of hemicelluloses does not even occupy a second place. This is due to a number of factors; namely, the fact that the latter are not so abundant quantitatively and are not of so universal occurrence as the former; a better understanding of the chemistry of cellulose and the confused state of our knowledge of the chemistry of hemicelluloses; the fact that the bacteria decomposing the hemicelluloses are not so specific in their metabolism as the cellulose-decomposing bacteria; the greater abundance of bacteria capable of bringing about the decomposition of hemicelluloses.

Hoppe-Seyler demonstrated in 1889 (10) that xylans are decomposed by bacteria found in river mud. The ability of many bacteria to decompose pentosans was later established by a number of other investigators; the same was known to be true of the degradation of mannans (16, 17); however, the literature is very meager concerning the ability of specific bacteria to attack galactans, so that it came to be recognized that these compounds are very resistant to microbial action.

The rôle of anaerobic bacteria in the decomposition of hemicelluloses in the digestive tract of ruminants (11, 12, 20) and in the manure pile (21) has been known for a long time. It has been shown (19), for example, that the microorganisms in the large intestine of guinea pigs convert xylan into acetic and butyric acids, in the proportion of 9 parts of the former to 1 part of the latter. The feces of animals was found (20) to contain bacteria which produce acetic, propionic, and butyric acids, as well as traces of optically inactive lactic acid, from hemicelluloses. Anaerobic bacteria were found to be able to attack not

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only pentosans but also methyl-pentosans (3). In the formation of silage by bacteria, a small amount of the pentosan was found (15) to be decomposed.

A number of aerobic bacteria were shown to be able to destroy hemicelluloses. This was true of the decomposition of the pentosan in green corn stover; *Bac. flavigena*, a cellulose-decomposing form, was found to be the most active organism. The specific decomposition of hemicelluloses by aerobic bacteria has been especially noted in the liquefaction of agar-agar, accompanied by hydrolysis; among the organisms capable of bringing about this process, one need only mention *Vibrio andoi* (1), *Bacillus nenckii* (4), *Bac. gelaticus* (8), *Microspira agar-liquefaciens* (9), *Bact. betae viscosum Panek*, and others (2, 16). Xylans were found to be decomposed by *Bac. mesentericus ruber* and *Bac. thermophilus grignoni* (7). In the infection of vegetables by *Bac. oleraceae* the hemicelluloses are known to be attacked. This is especially true of the pectins which are attacked by certain disease-producing bacteria, as in the case of the soft roots of carrots and other vegetables; the chemistry of the process is still imperfectly understood, although specific enzymes are known to be secreted by the organisms.

Mannans are attacked readily by *Bac. mesentericus vulgatus* (5) and by various soil bacteria (13, 16). Some of the bacteria produce tri-mannose from mannan (16); others produce laevudilin, a product of incomplete hydrolysis (13). Swartz (22) reported that the readiness with which salep (a source of mannan) disappears in the alimentary tract and is decomposed by fecal bacteria is similar to that of inulin. It is also interesting to note here that when uronic acids are decomposed by bacteria, the corresponding pentose may first be formed, as found by Salkowski and Neuberg (17) in the formation of *d*-xylose from glucuronic acid by bacteria.

It has been shown previously (24) that when plant residues are added to the soil, the hemicelluloses undergo rapid decomposition, not at a uniform rate, however. It has also been shown that numerous soil fungi and actinomyces are capable of decomposing large quantities of various hemicelluloses, liberating a part of the carbon as CO_2 and assimilating a part for the synthesis of microbial cell substance, as measured by the transformation of the nitrogen from an inorganic into an organic form. The galactans were found to be more resistant to decomposition than the mannans and xylans. The possibility has also been suggested (6) that certain hemicelluloses may be used as sources of energy by non-symbiotic nitrogen-fixing bacteria, without hydrolysis of the hemicellulose having to take place first by other bacteria, as found in the case of cellulose.

In order to study the decomposition of hemicelluloses by various typical soil bacteria, it was decided to isolate from the soil as many aerobic and anaerobic bacteria as possible; no attempt has been made, however, to make at the present time a detailed classification and identification of these organisms; the sole purpose was to test first the ability of these organisms to utilize these carbohydrates as sources of energy. Some of the cultures were saved for

further study, since they presented special interest, either because of the rapidity of hemicellulose decomposition that they brought about or because they formed special products of decomposition.

ISOLATION OF BACTERIA FROM SOIL

For the isolation of the bacteria, special media rich in hemicelluloses were employed. The cultures were isolated and tested first qualitatively, so as to determine which type of hemicellulose could supply the necessary energy for their life processes. Differences in specificity toward the various hemicelluloses were noted immediately. Some of the bacteria were able to decompose only one type of hemicellulose, others acted upon two different hemicelluloses, and still others were able to thrive on all the hemicelluloses studied. Table 1 gives a list of the bacteria isolated by the use of various carbohydrates as sources of energy and their ability to decompose the hemicellulose preparations as well as other carbohydrates. Attention should be called here to the fact that the mannan and galactan preparations were not pure hemicelluloses. The mere growth of an organism in the media containing these preparations is no proof at all that the organism is capable of using these as sources of energy. This is true especially of the galactan, as shown later in the quantitative studies.

DECOMPOSITION OF HEMICELLULOSES BY AEROBIC BACTERIA

For a more quantitative study of the decomposition of hemicellulose by bacteria, mannan prepared by the procedure outlined previously was at first employed. The tests were conducted in both sand and solution media. Each 100 cc. of solution or 100 gm. of sand contained the following ingredients:

	gm.
$(NH_4)_2HPO_4$	0.05
K_2HPO_4	0.05
$MgSO_4$	0.01
NaCl	0.01
$CaCl_2$	0.01
$FeSO_4$	0.001
Mannan preparation	0.50

The sand cultures were kept at 20 per cent moisture so as to allow optimum aerobic conditions for the activities of the bacteria. Inoculations were made after the materials had been sterilized; the cultures were then incubated at 27 to 28°C. for 6 weeks. At the end of the incubation period, the contents of the flasks were analyzed for total hemicellulose and for ammonia. Hydrolysis of the hemicellulose was effected by autoclaving with 2 per cent HCl under 15 pounds pressure for one-half hour. This method is possible only when there are no other polysaccharides present which would give reducing sugars upon hydrolysis. Ammonia determinations were made by distillation with magnesium oxide.

TABLE 1

List of aerobic and anaerobic bacteria isolated from soil on media containing various carbohydrates and their ability to decompose these carbohydrates

NUMBER OF CULTURE	AEROBISM OR ANAEROBISM	CARBOHYDRATE USED FOR ISOLATION	ABILITY TO UTILIZE CARBOHYDRATES				
			Glucose	Starch	Mannan	Galactan	Xylan
1	Aerobic	Pentosan	+	+	−	++	+*
2	Aerobic	Pentosan	+	+	+	++	−
3	Aerobic	Mannan	+	+	+	++	+
4	Aerobic	Mannan	+	+	++	+	+
5	Anaerobic	Mannan	+	+	++	+	−
6	Aerobic	Mannan	+	+	−	+	−
7	Aerobic	Galactan	+	−	−	+	−
8	Aerobic	Galactan	+	+	++	+	++
9	Aerobic	Galactan	+	+	+	++	+
10	Aerobic	Galactan	+	−	+	++	−
11	Aerobic	Galactan	+	+	+	++	−
12	Aerobic	Glucose	....	....	....	....	....
13	Aerobic	Pentosan	+	−	−	+	−
14	Aerobic	Mannan	+	−	−	+	−
15	Aerobic	Galactan	....	....	....	....	....
16	Aerobic	Glucose	....	....	....	....	....
17	Aerobic	Glucose	+	+	−	+	−
18	Aerobic	Pentosan	....	....	....	....	....
19	Aerobic	Pentosan	....	....	....	....	....
20	Aerobic	Pentosan	+	+	+	++	−
21	Aerobic	Mannan	....	....	....	....	....
22	Aerobic	Mannan	+	+	+	++	−
23	Aerobic	Galactan	....	....	....	....	....
24	Aerobic	Galactan	....	....	....	....	....
25	Aerobic	Mannan	....	....	....	....	....
27	Aerobic	Galactan	+	+	+	++	−
29	Aerobic	Pentosan	+	+	+	++	++
30	Aerobic	Pentosan	+	+	+	++	−
32	Aerobic	Xylan	....	....	....	....	....
34	Aerobic	Mannan	+	+	+	++	+
36	Aerobic	Galactan	+	+	+	++	+
37	Aerobic	Pentosan	+	+	+	+	+
38	Aerobic	Pentosan	+	+	++	+	−
39	Aerobic	Xylan	....	....	....	....	....
41	Aerobic	Mannan	+	+	+	+	+
42	Aerobic	Mannan	....	....	....	....	....
44	Aerobic	Galactan	+	+	++	+	−
45	Aerobic	Galactan	+	+	+	+	−
46	Aerobic	Xylan	....	....	....	....	....
55	Aerobic	Lignin	+	−	+	++	+
56	Aerobic	Lignin	+	−	++	++	+

* − = no growth.
\+ = positive growth.
++ = very good growth.

TABLE 1—*Concluded*

NUMBER OF CULTURE	AEROBISM OR ANAEROBISM	CARBOHYDRATE USED FOR ISOLATION	ABILITY TO UTILIZE CARBOHYDRATE				
			Glucose	Starch	Mannan	Galactan	Xylan
57	Aerobic	Lignin	+	+	++	+	+
58	Aerobic	Lignin	−	−	+	+	−
59	Aerobic	Lignin	+	−	+	+	+
60	Aerobic	Starch	+	+	+	++	+
61	Aerobic	Starch	+	+	++	+	−
62	Aerobic	Starch	+	+	+	++	+
63	Aerobic	Starch	+	+	++	+	−
64	Aerobic	Starch	+	+	+	+	++
65	Aerobic	Starch	+	+	+	++	−
66	Aerobic	Starch	+	+	+	+	−
67	Aerobic	Starch	+	−	+	++	−
68	Aerobic	Mannan	−	−	+	+	+
69	Aerobic	Mannan	+	+	+	++	+
70	Anaerobic	Mannan	−	−	++	+	−
71	Aerobic	Mannan	−	+	+	++	+
72	Aerobic	Mannan	+	−	++	+	+
73	Aerobic	Mannan	+	+	+	+	+
75	Aerobic	Galactan	....	....	....	....	....
77	Aerobic	Galactan	+	+	+	+	+
79	Aerobic	Galactan	....	....	....	....	....
80	Aerobic	Xylan	....	....	....	....	....
81	Aerobic	Xylan	....	....	....	....	....
82	Aerobic	Xylan	....	....	....	....	....
83	Aerobic	Xylan	....	....	....	....	....
84	Aerobic	Xylan	....	....	....	....	....
85	Aerobic	Xylan	....	....	....	....	....
86	Anaerobic	Galactan	+	+	+g†	+g	?
87	Anaerobic	Mannan	+	−	+g	+g	−
88	Anaerobic	Mannan	+	+	+	+	−
89	Anaerobic	Mannan	+	+	+g	+	?
90	Anaerobic	Mannan	+	−	−	+	?
91	Anaerobic	Mannan	+	+	+g	+g	−
92	Anaerobic	Xylan	+	+g	+	+	−
93	Anaerobic	Xylan	+	+	+	+	−
94	Anaerobic	Xylan	+	+	+	+	−
95	Anaerobic	Xylan	+	−	+g	+g	−
96	Anaerobic	Xylan	+	−	+g	+g	−
97	Anaerobic	Xylan	+	−	+g	+g	−

† g = gas evolution under anaerobic conditions.

The decomposition of mannan in sand media by pure cultures of bacteria is shown in table 2. All of the bacteria tested decomposed the mannan very actively, in some cases nearly 95 per cent of the hemicellulose disappearing within a period of 6 weeks. Even the least active organism, namely, Bacterium 83 decomposed nearly half of the mannan present, in this period of time. On the average, 33.5 parts of mannan were decomposed for each unit of nitro-

TABLE 2

The decomposition of mannan in sand medium by pure cultures of soil bacteria

INOCULUM	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	305.5	0	9.6	0	....
Bacterium 4	74.5	231.0	5.0	4.6	50.2
Bacterium 8	74.5	231.0	0.1	9.5	24.3
Bacterium 55	12.4	293.1	5.1	4.5	65.1
Bacterium 56	17.8	287.7	2.1	7.5	38.4
Bacterium 62	52.9	252.6	3.4	6.2	40.7
Bacterium 64	116.1	189.4	2.0	7.6	24.9
Bacterium 2	52.9	242.6	2.5	7.1	34.2
Bacterium 58	15.1	290.4	1.0	8.6	33.8
Bacterium 69	15.1	290.4	2.9	6.7	43.3
Bacterium 5	26.1	279.4	1.9	7.7	36.3
Bacterium 9	30.6	274.9	3.0	6.6	41.6
Bacterium 10	46.4	259.1	1.0	8.6	30.1
Bacterium 11	44.1	261.4	0.4	9.2	28.4
Bacterium 20	26.1	279.4	2.7	6.9	40.5
Bacterium 22	23.9	281.6	1.9	7.9	35.7
Bacterium 27	55.8	249.7	1.0	8.6	29.0
Bacterium 28	37.4	268.1	2.0	7.6	35.3
Bacterium 30	26.1	279.4	3.0	6.6	42.3
Bacterium 34	73.8	231.7	0.7	8.9	26.0
Bacterium 36	44.1	261.4	0.1	9.5	27.5
Bacterium 38	41.9	263.6	0.7	8.9	29.6
Bacterium 41	59.9	245.6	2.0	7.6	32.3
Bacterium 43	41.9	263.6	0.2	9.4	28.0
Bacterium 44	28.4	277.1	0.7	8.9	31.1
Bacterium 45	26.1	279.4	1.1	8.5	32.9
Bacterium 53	28.4	277.1	2.2	7.4	37.4
Bacterium 57	44.1	261.4	1.0	8.6	30.4
Bacterium 59	28.4	277.1	0.6	9.0	30.8
Bacterium 61	28.4	277.1	0.7	8.9	31.1
Bacterium 63	41.9	263.6	0.4	9.2	28.7
Bacterium 65	32.9	272.6	0.9	8.7	31.3
Bacterium 66	32.9	272.6	0.5	9.1	30.0
Bacterium 67	30.6	274.9	0.6	9.0	30.6
Bacterium 68	37.4	268.1	1.1	8.5	31.5
Bacterium 83	157.1	148.4	2.4	7.2	20.6
Bacterium 71	113.4	192.1	0.5	9.1	21.1
Bacterium 72	106.2	199.3	0.7	8.9	22.4
Bacterium 73	37.4	268.1	4.0	5.6	47.9
Bacterium 75	48.6	256.9	2.1	7.5	34.3
Bacterium 77	50.9	254.6	1.4	8.2	31.1
Bacterium 79	53.6	251.9	2.5	7.1	35.5
Bacterium 82	30.6	274.9	1.2	8.4	32.7

TABLE 3

The decomposition of mannan in solution medium by pure culture of soil bacteria

BACTERIUM	pH	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	5.4	382.5	0	9.6	0	....
Bacterium 4	5.2	270.5	112.0	6.2	3.4	32.9
Bacterium 8	5.2	334.8	47.7	7.6	2.0	23.9
Bacterium 55	5.2	265.5	117.0	5.2	4.4	26.6
Bacterium 56	5.2	338.0	44.5	8.0	1.6	28.3
Bacterium 62	5.2	280.8	101.7	5.0	4.6	22.1
Bacterium 64	5.0	213.8	168.7	1.6	8.0	21.1
Bacterium 2	5.2	310.5	62.0	7.0	2.6	23.9
Bacterium 58	5.2	334.8	47.7	7.6	2.0	23.9
Bacterium 69	5.1	340.7	31.8	8.1	1.5	21.2
Bacterium 5	5.2	326.7	55.8	8.4	1.2	46.5
Bacterium 9	5.0	156.6	225.9	1.6	8.0	28.2
Bacterium 10	5.4	352.4	30.1	7.4	2.2	13.7
Bacterium 11	5.4	310.5	72.0	3.0	6.6	10.9
Bacterium 20	5.4	349.2	33.3	6.4	3.2	10.4
Bacterium 22	5.2	286.7	95.8	5.8	3.8	24.7
Bacterium 27	5.2	326.7	55.8	7.8	1.8	31.0
Bacterium 28	5.2	318.6	63.9	5.6	4.0	16.0
Bacterium 30	5.2	318.6	63.9	8.4	1.2	53.3
Bacterium 38	5.2	297.0	85.5	7.2	2.4	35.5
Bacterium 41	5.2	310.5	72.0	5.2	4.4	16.4
Bacterium 44	5.2	305.1	77.4	6.8	2.8	27.6
Bacterium 45	4.8	162.0	220.5	0.4	9.2	23.9
Bacterium 53	5.2	46.4	336.1	2.4	7.2	46.7
Bacterium 57	5.2	329.4	53.1	6.2	3.4	15.6
Bacterium 59	5.2	307.4	75.1	4.8	4.8	15.6
Bacterium 61	5.2	268.2	114.3	4.8	4.8	23.8
Bacterium 63	5.2	351.5	31.0	7.0	2.8	11.1
Bacterium 65	5.0	118.4	264.1	1.2	8.4	31.4
Bacterium 66	4.8	96.8	285.7	0.0	9.6	29.8
Bacterium 67	4.6	186.3	196.2	0.2	9.4	20.9
Bacterium 68	5.2	284.9	97.6	4.8	4.8	20.3
Bacterium 83	4.8	92.3	290.2	0.2	9.4	30.9
Bacterium 71	5.2	360.0	22.5	7.4	2.2	10.2
Bacterium 72	5.2	297.0	85.5	4.4	5.2	16.4
Bacterium 73	5.2	346.1	36.4	7.4	2.2	16.5
Bacterium 75	5.2	348.3	34.2	6.8	2.8	12.2
Bacterium 77	5.2	273.6	108.9	6.8	2.8	38.9
Bacterium 79	5.2	348.3	34.2	7.2	2.4	14.3
Bacterium 82	5.2	297.0	85.5	5.8	3.8	22.5

gen assimilated by the bacteria. Considerable variation was, of course, noted, but because of the large number of cultures used this figure is very significant in respect to the nitrogen utilization by microörganisms decomposing the hemicelluloses. The average amount of mannan decomposed by the 42 different cultures of bacteria was 258.3 mgm., constituting 84.7 per cent of the hemicellulose added. It is to be expected, therefore, that since the mannan is so readily decomposed by bacteria in sand media, it will also be decomposed to a considerable extent in the soil by these microörganisms.

The decomposition of mannan in solution medium by pure cultures of bacteria is shown in table 3. A rather wide variation of the amounts of mannan decomposed is here noted. On the average, 103.5 mgm. of mannan, out of the original 382.5 mgm., were decomposed. For every part of nitrogen assimilated

TABLE 4

The decomposition of mannan in salep roots by pure cultures of soil aerobic bacteria

ORGANISM	pH	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	7.0	660.4	0	62.0	0	....
Bacterium 4	7.0	545.4	115.0	54.8	7.2	16.0
Bacterium 8	6.8	383.3	278.1	55.2	6.8	40.9
Bacterium 55	6.4	362.9	297.5	55.6	6.4	46.5
Bacterium 62	6.5	105.3	555.1	45.6	16.4	33.8
Bacterium 64	6.4	317.0	343.4	50.4	11.6	29.6
Bacterium 2	6.9	542.7	117.7	52.8	9.2	12.8
Bacterium 58	6.6	80.6	579.9	48.8	13.2	43.9
Bacterium 36	7.0	346.7	313.7	58.0	4.0	78.4
Bacterium 10	7.0	620.5	39.9	60.0	2.0	20.0
Bacterium 11	6.6	375.3	285.1	48.8	13.2	21.6
Bacterium 20	6.9	530.3	130.1	52.4	9.6	13.6
Bacterium 28	6.8	382.3	278.1	54.0	8.0	34.8

by the bacteria, 14.1 units of mannan were decomposed. The small amount of decomposition and the large amount of nitrogen consumed may be due to the fact that these organisms are essentially aerobic and conditions in the solution cultures were not so favorable for their development and for their physiological activities as they were in the sand cultures.

These results were obtained by the use of purified mannan preparations. In order to determine whether or not the extraction and preparation of the mannan had any influence upon its decomposition, the mannan in the natural state (salep root) was subjected to the activities of the bacteria showing the greatest amount of decomposition on the purified product. The results of this experiment are given in table 4.

The various aerobic bacteria destroyed varying amounts of mannan ranging

up to 87.8 per cent of the total mannan present. For every unit of nitrogen assimilated, the bacteria decomposed, on the average, 32.6 units of mannan.

TABLE 5

The decomposition of xylan in sand medium by pure cultures of soil aerobic bacteria

BACTERIUM	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	152.0	0	8.6	0	....
Bacterium 8	88.2	63.8	6.3	2.3	27.7
Bacterium 24	92.7	59.3	5.9	2.7	22.0
Bacterium 28	83.7	68.3	4.3	4.3	15.9
Bacterium 29	38.7	113.3	1.9	6.7	16.9
Bacterium 1	65.7	86.3	2.2	6.4	13.5
Bacterium 9	107.1	44.9	5.3	3.3	13.6
Bacterium 41	110.7	41.3	6.6	2.0	20.7
Bacterium 43	88.6	63.4	2.8	5.6	11.3
Bacterium 53	48.4	107.2	3.2	5.4	19.9
Bacterium 55	99.4	52.6	7.0	1.6	32.9
Bacterium 59	113.4	38.6	6.6	2.0	19.3
Bacterium 62	96.7	55.3	5.6	3.0	18.4
Bacterium 68	113.4	38.6	5.9	2.7	14.3

TABLE 6

Decomposition of xylan in solution media by pure cultures of soil aerobic bacteria

BACTERIUM	pH	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm.*	*mgm*	*mgm*	
Control	6.2	287.3	0	8.6	0	....
Bacterium 4	6.2	271.8	15.5	8.2	0.4	38.8
Bacterium 8	6.2	205.8	81.5	3.6	5.0	16.3
Bacterium 24	6.2	156.2	131.1	3.6	5.0	26.2
Bacterium 28	6.2	238.2	49.1	7.6	1.0	49.1
Bacterium 29	6.2	138.6	148.7	3.6	5.0	29.7
Bacterium 64	6.2	138.6	148.7	4.6	4.0	37.2
Bacterium 1	6.2	267.6	19.7	8.2	0.4	49.3
Bacterium 3	6.2	258.6	26.7	8.2	0.4	66.8
Bacterium 34	6.2	275.4	11.9	8.2	0.4	24.8
Bacterium 36	6.2	278.4	8.9	8.0	0.6	14.9
Bacterium 43	5.8	157.8	129.5	2.8	5.8	22.3
Bacterium 53	6.2	241.8	45.5	8.4	0.2	....

It is to be remembered that the nitrogen utilization values in the case where the natural materials were used are not as exact and reliable as they are in the

experiments where the purified products were employed, because in the former case the organisms may have attacked other substances, while the nitrogen in the salep root itself may have been preferred to the nitrogen added.

The experiments with the xylan preparation followed the same plan as outlined for the mannan. The flasks were inoculated with organisms which had

TABLE 7

The decomposition of xylan in corn cobs in solution media by pure cultures of soil aerobic bacteria

ORGANISM	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	405.5	0	30.0	0	....
Bacterium 43	368.8	36.7	28.0	2.0	18.4
Bacterium 64	372.1	33.4	29.2	0.8	41.8
Bacterium 8	241.9	163.6	26.8	3.2	51.1
Bacterium 28	250.6	154.9	27.6	2.4	64.5
Bacterium 53	271.6	133.9	24.4	5.6	23.9
Bacterium 1	167.9	237.6	24.0	6.0	39.6

TABLE 8

Decomposition of galactan in solution media by pure cultures of soil aerobic bacteria

ORGANISM	GALACTAN		AMMONIA-NITROGEN	
	Found	Decomposed	Found	Assimilated
	mgm.	*mgm.*	*mgm.*	*mgm.*
Control	306.9	0	9.3	0
Bacterium 1	144.9	162.0	8.3	1.0
Bacterium 2	176.4	130.5	9.0	0.3
Bacterium 4	173.7	133.2	9.0	0.3
Bacterium 9	157.5	148.4	8.7	0.6
Bacterium 11	229.5	77.4	8.3	1.0
Bacterium 17	269.1	37.8	9.0	0.3
Bacterium 20	252.0	54.9	9.0	0.3
Bacterium 22	261.9	45.0	8.7	0.6
Bacterium 41	269.1	37.8	8.0	1.3
Bacterium 27	229.5	77.4	8.7	0.6
Bacterium 34	265.5	41.4	8.0	1.3

shown, in previous trials, that they are capable of decomposing the xylan. The incubation period was 6 weeks at a temperature of 27° to 28°C. The methods of analysis were the same as those employed in the experiment with mannan.

The decomposition of the xylan in sand media by pure cultures of aerobic bacteria is reported in table 5. In only two cases was more than two-thirds of

the xylan destroyed. The average amount of xylan decomposed was 42.1 per cent. In this study, 18.9 parts of the xylan were utilized per unit of nitrogen assimilated. There was a close agreement in these values for the various bacteria.

The decomposition of the xylan in solution media by the aerobic bacteria is shown in table 6. On the average, 23.7 per cent of the xylan was decomposed, but in no case did the amount destroyed reach much more than 50 per cent of the total material originally present. In solution media the bacteria used 1 part of nitrogen for every 31.3 parts of xylan decomposed.

The ability of the bacteria to decompose xylan in the natural state was also determined. Since the xylan preparation had been obtained from corn cobs, this source of material was employed to study the decomposition of xylan as

TABLE 9

Decomposition of galactan in Irish moss in solution media by pure cultures of soil aerobic bacteria

ORGANISM	GALACTAN		AMMONIA-NITROGEN		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	382.3	0	50.0	0	....
Bacterium 2	263.0	119.3	46.0	4.0	29.8
Bacterium 9	321.8	60.5	46.0	4.0	15.1
Bacterium 11	321.8	60.5	45.2	4.8	12.6
Bacterium 17	291.1	91.2	46.0	4.0	22.8
Bacterium 20	287.1	95.2	46.4	3.6	26.4
Bacterium 22	344.0	38.3	....	...	....
Bacterium 41	360.2	22.1	....	...	....
Bacterium 27	287.1	95.2	48.0	2.0	47.6
Bacterium 34	271.6	110.7	43.2	6.8	16.3

it exists in nature. It should be remembered that xylan makes up only about 30 per cent of the total constituents of the corn cobs. Nevertheless, it was believed that the study of the activities of xylan-decomposing organisms upon the ground corn cobs would aid in the formulation of a clearer conception of the utilization of xylan by these bacteria. These studies were carried out in solution media and the results are given in table 7.

The bacteria decomposed, on the average, 31.2 per cent of the xylan present. This is about 8 per cent higher than the decomposition of the purified xylan under the same conditions. Nearly 40 parts of xylan were decomposed per unit of nitrogen assimilated.

The experiments dealing with the decomposition of galactan by various bacteria were carried out in exactly the same manner as those reported for the mannan and xylan. The galactan was found to be more resistant to decom-

position by microörganisms than were the other two hemicelluloses. Variation among bacteria in their ability to decompose the galactan was more marked than in the case of the other hemicelluloses. The results of the decomposition of the galactan in solution media by pure cultures of aerobic bacteria are given in table 8. In four cases, about half of the galactan was decomposed.

TABLE 10

Decomposition of mannan in salep roots by pure cultures of anaerobic bacteria

ORGANISM	pH	MANNAN		NH_3-N		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	6.6	770.0	0	114.4	0	....
Bacterium 5	5.8	411.5	358.5	104.4	10.0	35.9
Bacterium 70	6.2	171.7	598.3	108.0	6.4	93.5
Bacterium 86	6.2	688.0	82.0	109.6	4.8	17.1
Bacterium 87	4.8	155.5	614.5	106.0	8.4	73.2
Bacterium 88	4.8	238.7	531.3	106.4	8.0	66.4
Bacterium 89	6.3	757.1	12.9	111.6	2.8	....
Bacterium 90	4.8	128.5	641.5	107.2	7.2	89.1
Bacterium 91	5.4	429.8	340.2	112.8	1.6	21.3
Bacterium 92	5.2	341.3	428.7	108.4	6.0	71.5
Bacterium 93	6.2	526.0	244.0	102.4	12.0	20.3
Bacterium 94	5.6	388.8	381.2	110.4	4.0	95.3
Bacterium 95	5.4	411.5	358.5	107.6	6.8	52.7
Bacterium 96	5.2	429.8	340.2	109.6	4.8	70.9

TABLE 11

Composition of the gases produced by Bacterium 70 in the decomposition of mannan in salep roots

CONSTITUENT	(a)*	(b)	(c)
	per cent	*per cent*	*per cent*
Carbon dioxide (CO_2)	0.2	4.6	8.0
Oxygen (O_2)	3.9	2.2	2.0
Hydrogen (H_2)	2.7	0.0	0.0
Methane (CH_4), carbon monoxide, (CO), nitrogen (N_2), etc	93.2	93.2	90.0

* (*a*) = first gas collection, 2,225 cc.; (*b*) = second, 2,350 cc.; (*c*) third, 2,500 cc.

The remaining cultures decomposed a much smaller amount of this carbohydrate, so that, on the average, only 23.2 per cent of the galactan disappeared, as a result of bacterial action. It is interesting to note that the amounts of inorganic nitrogen used by the bacteria in the decomposition of the galactan were very small, possibly because of the utilization of some of the nitrogen found in the galactan preparation.

In order to compare the influence of treatment in the process of preparation of the galactan upon its decomposition by bacteria, the natural product, namely Irish moss, was employed as a source of energy for these bacteria. The results of these studies, shown in table 9, are similar to those obtained with the purified preparation. The resistance of the galactan to decomposition by various microörganisms is further brought out in this experiment. The bac-

TABLE 12

Composition of the gas produced by Bacterium 5 in the decomposition of the mannan in salep roots

CONSTITUENT	PER CENT OF TOTAL
Carbon dioxide (CO_2)	20.4
Oxygen (O_2)	0.4
Hydrogen (H_2)	45.4
Methane, carbon monoxide, nitrogen, etc.	33.8

TABLE 13

Decomposition of galactan in Irish moss by pure cultures of anaerobic bacteria

ORGANISM	GALACTAN		NH_3-N		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
Control	531.4	0	112.8	0	....
Bacterium 5	447.1	84.3	109.2	3.6	23.5
Bacterium 70	453.6	77.8	108.8	4.0	19.5
Bacterium 86	No growth				
Bacterium 87	459.0	72.4	109.6	3.2	22.6
Bacterium 88	435.2	96.2	108.0	4.8	20.0
Bacterium 89	465.5	65.9	109.6	3.2	20.6
Bacterium 90	441.7	89.7	107.6	5.2	17.3
Bacterium 92	No growth				
Bacterium 93	459.0	72.4	105.2	7.6	9.5
Bacterium 94	441.7	89.7	111.2	1.6	56.2
Bacterium 95	No growth				
Bacterium 96	No growth				
Bacterium 97	No growth				

teria were able to decompose only about 20.0 per cent of the galactan in the Irish moss. For every unit of nitrogen assimilated about 20 parts of galactan were decomposed.

DECOMPOSITION OF HEMICELLULOSES BY ANAEROBIC BACTERIA

In addition to the aerobic bacteria, a number of anaerobes were tested for their hemicellulose decomposing capacity. The anaerobic organisms were obtained from the soil by the use of media containing the various hemicelluloses

as sources of energy. They were allowed to develop in an atmosphere free from atmospheric oxygen. Practically all of these bacteria were gas-formers when grown in solutions containing the specific hemicelluloses. Microscopic examination showed that they were all spore-forming rods of various sizes.

It was very difficult to grow these organisms on the purified hemicellulose preparations. In order to avoid great variation in the results, the bacteria were grown on the natural materials rich in hemicelluloses in liquid media.

TABLE 14

Evolution of carbon dioxide from mannan in sand medium by pure cultures of bacteria

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION						
	2	4	6	11	18	28	42
Control	0.1	0.3	0.7	2.0	3.5	4.0	5.2
Bacterium 2	1.7	18.0	36.7	48.9	55.4	61.0	72.2
Bacterium 4	3.0	3.1	6.2	16.6	30.2	42.3	55.2
Bacterium 8	8.3	23.0	36.6	44.6	53.7	65.7	81.7
Bacterium 55	16.6	25.8	42.7	54.5	68.1	81.1	98.3
Bacterium 56	8.4	26.5	35.5	43.6	49.8	52.0	64.7
Bacterium 58	9.2	27.6	34.8	41.5	49.0	56.3	67.6
Bacterium 62	12.6	27.2	39.6	54.6	73.0	83.3	88.2
Bacterium 64	18.4	35.8	47.8	55.1	61.4	68.7	80.7
Bacterium 69	16.4	29.8	38.6	44.3	51.2	65.6	83.1

TABLE 15

Evolution of carbon dioxide from mannan in salep roots by aerobic bacteria

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION						
	8	11	13	15	18	24	30
Bacterium 8	16.2	30.1	40.9	50.3	57.8	69.6	73.6
Bacterium 55	6.7	14.0	18.7	23.9	29.6	39.9	47.4
Bacterium 58	8.5	25.8	35.9	43.8	51.8	74.7	94.7
Bacterium 62	15.5	33.6	43.5	51.1	57.3	67.2	71.0

The decomposition of the mannan of salep root by pure cultures of anaerobic bacteria is shown in table 10. The media containing the necessary mineral nutrients were inoculated and incubated for 6 weeks. Only three of the organisms decomposed less than 45 per cent of the mannan present in the medium, whereas in one case as much as 83 per cent of the hemicellulose was decomposed. Large quantities of organic acids were produced in some cases. Considerable variation was observed in the nitrogen utilization, and, therefore, too much emphasis should not be placed upon the results. It is quite probable that, in addition to the mannan, other compounds of the salep root were de-

composed in some instances. This is shown by the fact that *Bacterium 89*, which decomposed the least amount of mannan, had produced a very heavy growth in the culture flask; this also accounts for the comparatively high consumption of the available nitrogen. A detailed study was made of the gases produced by *Bacterium 5* and *Bacterium 70*. The cultures were grown in liter flasks and the gas was collected over saturated solutions of NaCl. The gas produced by *Bacterium 70* was collected in three lots, as follows: (*a*) 2,225 cc.; (*b*) 2,350 cc.; and (*c*) 2,500 cc. The composition of these gases is given in table 11.

It is interesting to note that hydrogen was formed only at the beginning of the fermentation period. In no case was the sum of the methane, carbon monoxide, and nitrogen below 90 per cent of the total gas evolved. There was a steady increase in the carbon dioxide with a decrease in the oxygen.

TABLE 16

Evolution of carbon dioxide from xylan by pure cultures of bacteria

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION				
	4	6	18	28	42
Control	0.3	0.8	2.5	3.8	5.1
Bacterium 1	1.7	2.5	17.5	35.7	43.4
Bacterium 3	1.0	1.7	8.0	11.3	14.7
Bacterium 8	5.8	7.8	10.6	12.6	14.7
Bacterium 9	2.9	7.1	20.0	25.7	33.1
Bacterium 24	12.5	22.3	35.9	36.7	37.9
Bacterium 28	3.6	5.6	7.1	7.8	9.1
Bacterium 29	13.6	24.4	34.7	37.2	49.9
Bacterium 64	1.2	11.9	22.6	25.6	28.7

Bacterium 5 produced only 1,050 cc. of gas. The composition of this gas is given in table 12. The analysis of this gas shows that the processes of decomposition brought about by the two bacteria are widely different in character. Practically two-thirds of the gas produced by *Bacterium 5* consisted of carbon dioxide and hydrogen. Since almost half of the gas consists of hydrogen, this may be spoken of as essentially a hydrogen fermentation process. The amount of oxygen in this case is negligible. A detailed study of the morphological characters and physiological activities of this organism will be published later.

The decomposition of the galactan in the Irish moss by pure cultures of anaerobic soil bacteria is shown in table 13. The medium consisted of 1 per cent galactan in the form of Irish moss, added to a mineral solution. The decomposition process was allowed to proceed for 6 weeks. The pH of the solutions remained practically constant. This is no proof that organic acids were not produced in the decomposition of the galactan, but may be merely a

result of the buffering properties of the ammonium phosphate in the mineral solution and of the galactan itself. However, it indicated that only small amounts, if any, of organic acids were produced. In no case was more than 18 per cent of the galactan decomposed. This is in accord with the resistance which the galactan was shown to possess toward the action of microörganisms in the other experiments.

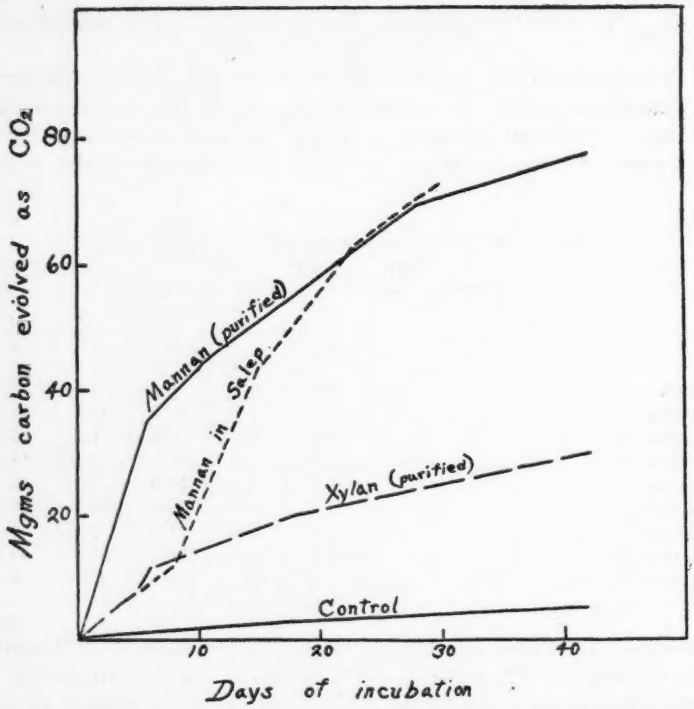

FIG. 1. COMPARISON OF THE RATE OF DECOMPOSITION OF CERTAIN HEMICELLULOSES BY AEROBIC BACTERIA

EVOLUTION OF CO_2 IN THE DECOMPOSITION OF HEMICELLULOSES

In order to ascertain the relative speed of decomposition of the various hemicelluloses by microörganisms, the carbon dioxide evolved was measured at short intervals over an extended period of time. The culture medium was identical with that outlined for the sand medium in the mannan experiment. Carbon dioxide-free air was drawn through the culture flasks to displace the carbon dioxide in the flasks and the latter was absorbed in $N/6$ solution of bar um hydroxide. The carbon dioxide produced was measured by frequent titrations of the excess barium hydroxide which served as a measure of the rate of decomposition.

Table 14 shows the carbon dioxide evolved by aerobic bacteria in the decomposition of mannan. The total amounts of carbon dioxide liberated, measured as carbon, ranged from 55.2 to 98.3 mgm. The various bacteria were found to exhibit different rates of decomposition. Some of them decomposed the mannan rapidly at the beginning and then more slowly; some acted slowly at first and more rapidly later, whereas others maintained practically an even rate of decomposition throughout the incubation period.

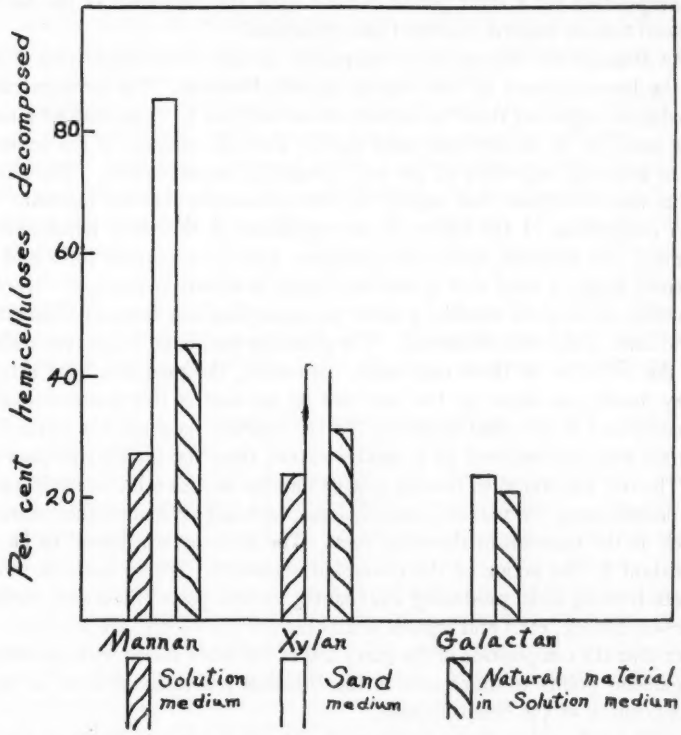

FIG. 2. EXTENT OF DECOMPOSITION OF VARIOUS HEMICELLULOSES BY AEROBIC BACTERIA

Following the same plan of experiment, the evolution of carbon dioxide in the decomposition of natural mannan (salep root) by certain bacteria was studied. It is certain that not all the carbon dioxide evolved came from the hemicellulose in the salep, but the mannan content was so large in proportion to the other constituents and the microörganisms have shown their abilities to decompose the mannan so readily, that one is safe in assuming that most of the carbon came from the decomposition of the mannan. The results of this experiment are given in table 15. The bacteria were active over an extended

period of time. This is shown by the fact that the rate of decomposition did not decrease very much toward the end of the incubation period.

The rate of decomposition of the xylan preparation obtained from corn cobs by various aerobic bacteria was also studied. The plan of the experiment was identical with that employed in the case of the mannan. Table 16 shows the accumulative evolution of carbon dioxide from xylan in the sand medium by pure cultures of the aerobic bacteria. A few organisms showed very rapid decomposition for a short period of time while the remainder of the bacteria showed a more uniform course of decomposition.

An attempt has been made to summarize the rate of decomposition of some of the hemicelluloses by the various aerobic bacteria. The average carbon dioxide evolution by these bacteria is shown in figure 1. The purified mannan was found to be decomposed more rapidly than the mannan in the untreated plant material, especially in the early stages of decomposition. The purified xylan was decomposed less rapidly and less extensively than the mannan.

A comparison of the extent of decomposition of the three hemicelluloses; namely, the mannan, xylan, and galactan, both in a purified form and in a natural state, in sand and in solution media is shown in figure 2. Since the bacteria tested were aerobic, greater decomposition was brought about in the sand than in the solution media. The galactan was found to be very resistant to the activities of these organisms. However, the mannans were attacked very readily, as shown by the fact that 85 per cent of this hemicellulose was decomposed in the sand medium. In the solution medium, the natural materials were decomposed to a greater extent than the purified preparations.

The soil was found to contain a large number of anaerobic bacteria capable of decomposing the various hemicelluloses, especially the unpurified materials, such as the mannan in the salep root. The galactan was found to be most resistant to the action of the anaerobic organisms. These bacteria were all spore-forming rods, producing abundantly various gases (hydrogen, methane, carbon dioxide, etc.) and organic acids (butyric, propionic, acetic, etc.). The fact that the composition of the gases and of the acids varied with the different organisms points to differences in the chemical processes involved in the decomposition of the hemicelluloses.

SUMMARY

Investigations dealing with the decomposition of various hemicelluloses by aerobic and anaerobic bacteria are reported.

Many aerobic bacteria were found to be able to decompose hemicelluloses, the differences observed being both qualitative and quantitative in nature.

Galactan was found to be more resistant to decomposition by aerobic bacteria than mannan and xylan.

In the case of aerobic bacteria, both the nature of the hemicellulose and the environmental conditions influenced markedly the quantities of hemicellulose decomposed.

Chemical purification appears to render hemicelluloses more resistant to decomposition. This is especially true of the anaerobic bacteria.

Organic acids were produced both by aerobic and anaerobic bacteria in the decomposition of hemicelluloses.

The anaerobic bacteria produced different gases in the decomposition of the hemicelluloses.

GENERAL SUMMARY

The results presented in these papers point definitely to the fact that the soil contains an abundant flora or fungi, actinomyces, aerobic and anaerobic bacteria, capable of decomposing various hemicelluloses.

The microörganisms decomposing the hemicelluloses are not so specific as those bacteria and fungi which are capable of decomposing cellulose. It is true, of course, that some organisms attack preferably one type of hemicellulose and some another; some of the hemicelluloses, like the galactans, are more resistant to decomposition than others, like the mannans, and probably also more than even the true cellulose. However, the decomposition of the most common hemicelluloses, such as the xylans and the other pentosans as well as the mannans, is not limited to any one special group or few specific types of bacteria and fungi, but can be brought about by a great variety of organisms found among a number of different types which are of common occurrence in nature.

As a result of this lack of specificity among the fungi and bacteria capable of attacking hemicelluloses, no attempt has been made to describe new types of organisms bringing about the decomposition of these carbohydrates, since hundreds of species could thus be readily isolated and described. The desirability of describing all of these species was questioned, especially because most of them did not represent any special physiological or morphological types. Of course, one cannot deny the existence of certain specific bacteria capable of attacking one type of hemicellulose in preference to others, as was found to be the case of certain pectin dissolving bacteria, agar-liquefying bacteria, bacteria decomposing capsular polysaccharides, etc. If such specific organisms are isolated, especially if they represent groups of possible economic importance, they should, of course, be studied and described in detail. This was found to be the case of a group of anaerobic bacteria which, as a result of the production of an abundance of organic acids and gases in the decomposition of hemicelluloses, may represent certain interesting phases of microbial physiology and possible economic utilization. These cultures have been reserved for a more extended study in the future.

The microörganisms bringing about the decomposition of the hemicelluloses require available sources of nitrogen for the synthesis of their microbial cell substance, just as cellulose-decomposing bacteria do. The ratio between the hemicellulose decomposed and the nitrogen consumed is about 30 to 1 with a variation of 20 to 40 to 1. In this respect, they resemble the fungi and repre-

sent an interesting phase of transformation of organic matter in soil, and formation of soil organic matter or soil humus.

The study of the transformation of uronic acid complexes in the decomposition of hemicelluloses by microörganisms is reserved for a future publication.

REFERENCES

(1) AOI, K. 1924 Über eine neue agarzersetzende Bodenbakterienart. *Centl. Bakt.* (II) 63: 30–32.

(2) AOI, K., AND ORIKURA, J. 1928 On the decomposition of agar, xylan, etc. and the sugars related to these hemicelluloses by *Vibrio Andoi* (n. sp.). *Centbl. Bakt.* (II) 74: 321–332.

(3) BERKELEY, C. 1920 Pentosan- and Methylpentosan-splitting enzymes of *Macrocystis pyrifera*. *Proc. Amer. Soc. Biol. Chem., Jour. Biol. Chem.* 41: LVI.

(4) BIERNACKI, W. 1911 *Bacterium Nenckii* Biern., ein neuer den Agar verflüssigender Mikroorganismus. *Centbl. Bakt.* (II) 29: 166–169.

(5) BROWNE, C. A. 1904 The hydrolytic products of sugar-cane fiber. *Jour. Amer. Chem. Soc.* 26: 1221–1235.

(6) DIEHM, R. 1930 The utilization of various hemicelluloses as sources of energy for nitrogen-fixing bacteria. *Proc. 2nd. Internatl. Cong. Soil Sci. Third Comn.*

(7) DUPONT, M. C. 1902 Sur les fermentations aerobies du fumier. *Compt. Rend. Acad. Sci.* (Paris) 134: 1449.

(8) GRAN, H. 1902 Studien über Meeresbakterien. II. Über die Hydrolyse des Agars durch ein neues Enzym, die Gelase. Bergens Museum Aarb. No. 2. [*Centbl. Bakt.* (II) 9: 562].

(9) GRAY, P. H., AND CHALMERS, C. H. 1924 On the stimulating action of certain organic compounds on cellulose decomposition by means of a new organism that attacks both cellulose and agar. *Ann. Appl. Biol.* 11: 324–338.

(10) HOPPE-SEYLER, F. 1889 Ueber die Bildung von Huminsubstanzen, ihre Entstehung und ihre Eigenschaften. *Ztschr. physiol. Chem.* 13: 66–121.

(11) LOHRISCH, HANS. 1909 Der Vorgang der Cellulose- und Hemicellulosen-verdauung beim Menschen und der Nährwerth dieser Substanzen für den menschlichen Organismus. *Ztschr. exp. Path.* 5: 478–539.

(12) MCCOLLUM, E. V., AND BRANNON, W. A. 1909 The disappearance of pentosans from the digestive tract of the cow. *Jour. Amer. Chem. Soc.* 31: 1252–1260.

(13) MAYEDA, M. 1922 Preliminary communication on mannase and laevidulinase. *Jour. Biochem.* 1: 131–137, 1922; Diss Tokyo.

(14) OHTSUKI, T. 1928 Konjak Mannan. *Acta Phytochim.* (Japan) 4: 1–39.

(15) PETERSON, W. H., FRED, E. B., AND VERHULST, J. H. 1921 The destruction of pentosans in the formation of silage. *Jour. Biol. Chem.* 46: 329–338.

(16) PRINGSHEIM, H. Über den fermentativen Abbau der Hemizellulosen. *Ztschr. Physiol. Chem.* 80: 376–382.

(17) SALKOWSKI, E., AND NEUBERG, C. 1902, 1903 Zur Frage der biochemischen Verwandlung von Kohlehydraten der *d*-Reihe in solche der *l*-Reihe. *Ztschr. Physiol. Chem.* 36: 261 (1902); 37: 464–466.

(18) SAWAMURA, S. 1902 On the liquefaction of mannan by microbes. *Coll. Agr. Tokyo Bul.* 5: 259–262.

(19) SEILIÈRE, G. 1910 Observations sur la composition et la digestilulité de quelques tissus végetaux. *Comp. Rend. Soc. Biol.* 68: 989–991.

(20) SHIMIZU, T. 1921 Über das Schicksal einiger Polysaccharide im Verdauungskanal bei Säugetieren. *Biochem. Ztschr.* 117: 227–240.

(21) SJOLLEMA, S., AND DEWILDT, J. C. DER. 1907 Experiments on the decomposition and fertilizing value of manure. *Verslag. Landbouwk. Onderzoek. Rijksl.* 1: 21–68. (*Exp. Sta. Rec.* 20: 721).

(22) SWARTZ, M. D. 1911 Nutrition investigations on the carbohydrates of lichens, algae and related substances. *Trans. Conn. Acad. Arts and Sci.*, 16: 247–382.

(23) VERHULST, J. H., PETERSON, W. H., AND FRED, E. B. 1923 Distribution of pentosans in the corn plant at various stages of growth. *Jour. Agr. Res.* 23: 655–663.

(24) WAKSMAN, S. A., AND DIEHM, R. A. 1931 On the decomposition of hemicelluloses by microörganisms. *Soil Sci.* 32: 73-95.

STUDIES ON THE TRANSFORMATIONS OF IRON IN NATURE: III. THE EFFECT OF CO_2 ON THE EQUILIBRIUM IN IRON SOLUTIONS

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In a former publication in this series (11), conditions of equilibrium were considered in iron solutions upon which the following restrictions were placed:

First, that ferrous iron underwent reversible oxidation to ferric iron according to the equation:

$$4\,\overset{\cdot\cdot}{Fe} + O_2 + 2\,\dot{H} = 4\,\overset{\cdot\cdot\cdot}{Fe} + 2\,\overline{OH}.$$

Second, that the solution was saturated with respect to the ferric iron in accordance with the equation:

$$[A_{\overset{\cdot\cdot\cdot}{Fe}}]\,[A_{\overline{OH}}]^3 = K$$

With these restrictions and under atmospheric pressure of oxygen the following relationship was deduced:

$$\frac{[A_{\overset{\cdot\cdot}{Fe}}]}{[A_{\dot{H}}]^2} = C$$

Solutions of ferrous sulfate were allowed to remain in contact with air for 4 months. At the end of this time the concentration of $Fe^{\cdot\cdot}$ was obtained from a determination of the total amount of iron present. The solutions were very dilute so that this was assumed to be equal to the activity of the ferrous ions. The activity of the hydrogen ion was determined from pH measurements. Under these conditions the average value of the foregoing ratio in a series of tests was found to be 4.8×10^8.

In the second article of the series (22) the rôle of microorganisms in the transformations of iron in nature was considered in the light of the equations developed. In attempting to evaluate the rôle of microorganisms it became apparent that CO_2 is of major importance.

In order that the influence of CO_2 may be better understood, it is necessary to see what effect this gas may have upon the aforementioned equilibrium. In the foregoing deductions, it was assumed that the solutions containing ions of iron would undergo a reversible oxidation when exposed to atmospheric oxy-

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gen. Because of the peculiarities of molecular oxygen when employed as an oxidizing agent, this assumption may not hold. To get a better picture of the true state of affairs it becomes necessary to avoid this assumption.

Equilibrium conditions can then be deduced by making use of the methods employed by Clark (4) and Kolthoff and Furman (13). They employed the fundamental equation of Nernst (1889),

$$E = -\frac{RT}{NF} ln \frac{P'}{p},$$

where E is the potential difference between the metal and the solution, T the absolute temperature, N the valence of the ion, and F the Faraday, i.e., the quantity of electricity that is associated with one gram-equivalent, viz., 96,500 coulombs. R and F are constants, the values of which are known. P' is the solution pressure of the metal, and p is the osmotic pressure of the metallic ions. Transposing the natural logarithms to the Briggsian, the following is obtained:

$$E = -\frac{0.0591}{N} \log \frac{P'}{p}$$

From this equation, they deduce the following:

$$E = +\ 0.0591 \log \frac{A}{A_e}, \qquad (A)$$

where A_e represents the electronic activity of the solution, a value which is a measure of the oxidation-reduction potential of the system. A is a constant so that equation (A) can be rewritten as follows:

$$E = A' - 0.0591 \log A_e \qquad (B)$$

In a solution where ferrous iron is oxidized reversibly to ferric iron, the changes can be indicated by the following equation:

$$\overset{\cdots}{\mathrm{Fe}} + e = \overset{\cdot\cdot}{\mathrm{Fe}}.$$

According to the mass law, this will give

$$\frac{[A_{\mathrm{Fe}^{\cdots}}]\,[A_e]}{[A_{\mathrm{Fe}^{\cdot\cdot}}]} = K$$

or

$$A_e = K \frac{[A_{\mathrm{Fe}^{\cdot\cdot}}]}{[A_{\mathrm{Fe}^{\cdots}}]}$$

Upon substituting this value of A_e in equation (B) there is obtained

$$E = A' - 0.0591 \log K \frac{[A_{\mathrm{Fe}^{\cdot\cdot}}]}{[A_{\mathrm{Fe}^{\cdots}}]}$$

or

$$E = A' - 0.0591 \log K + 0.0591 \log \frac{[A_{Fe}^{\cdot\cdot\cdot}]}{[A_{Fe}^{\cdot\cdot}]}.$$

The term $(A' - 0.0591 \log K)$ is constant for this system and can be called E_o. Then,

$$E_o = E + 0.0591 \log \frac{[A_{Fe}^{\cdot\cdot\cdot}]}{[A_{Fe}^{\cdot\cdot}]} \qquad (C)$$

Putting the E_o of the normal hydrogen electrode equal to O and using the sign convention adapted by the American Electrochemical Society, Peters (19) has determined the value of E_o ($Fe^{\cdot\cdot\cdot} - Fe^{\cdot\cdot}$) to be +0.743. Forrester (7) gives the value of +0.714. This value is accepted by Clark (4), and Kolthoff and Furman (13), and is probably the more accurate. Using this value we can then write.

$$E_h = + 0.714 + 0.0591 \log \frac{[A_{Fe}^{\cdot\cdot\cdot}]}{[A_{Fe}^{\cdot\cdot}]} \qquad (D)$$

By means of this equation it is possible to calculate the oxidation-reduction value of the solutions containing varying amounts of ferrous and ferric iron. It is also possible by means of this equation to determine the stable ratio of these two ions in solutions when the oxidation potential is fixed by some other system.

In a similar manner we can develop the equation of the oxidation-reduction potential of a system in which oxygen is in equilibrium with oxygen combined in the form of water, according to the equation:

$$O_2 + 4\,\dot{H} + 4\,e = 2\,H_2O.$$

By applying the mass law, we get the following:

$$A_e = \frac{K}{[A_{O_2}]^{\frac{1}{4}}\,[A_{\dot{H}}]}$$

Substituting this value of A_e in equation (B) we have

$$E = A' - 0.0591 \log K + 0.0591 \log [A_{O_2}]^{\frac{1}{4}}\,[A_{\dot{H}}]$$

or

$$E = E_o + 0.0591 \log [A_{O_2}]^{\frac{1}{4}}\,[A_{\dot{H}}] \text{ at } 25°C.$$

$E_{o(O_2 - H_2O)}$ has been calculated by G. N. Lewis (14) from the heats of formation of water from the elements. He obtained the value for E_o of 1.227 referred to the hydrogen scale. This value was obtained by using values for the heats of formation obtained from three independent sources. The value

should therefore be correct for systems in which there is a perfectly reversible exchange of electrons between the oxygen combined in water and molecular oxygen. We can then write:

$$E_h = + 1.227 + 0.0591 \log [A_{O_2}]^{\frac{1}{4}} [A_{\dot{H}}] \qquad (E)$$

If we assume that ferrous ions were oxidized reversibly by molecular oxygen, we can, by means of equations (*D*) and (*E*), calculate the equilibrium constant of this reaction.

$$4\ \ddot{Fe} + O_2 + 4\ \dot{H} = 4\ \dddot{Fe} + 2\ H_2O.$$

From this, the mass law will give

$$K = \frac{[A_{\ddot{Fe}}] [A_{O_2}]^{\frac{1}{4}} [A_{\dot{H}}]}{[A_{\dddot{Fe}}]}$$

At equilibrium the oxidation potentials of the systems indicated in equations (*D*) and (*E*) must be equal. Therefore,

$$E = + 0.714 + 0.0591 \log \frac{[A_{\dddot{Fe}}]}{[A_{\ddot{Fe}}]} = + 1.227 + 0.0591 \log [A_{O_2}]^{\frac{1}{4}} [A_{\dot{H}}].$$

From this,

$$- \log K = 8.64 = \log \frac{[A_{\dddot{Fe}}]}{[A_{\ddot{Fe}}] [A_{O_2}]^{\frac{1}{4}} [A_{\dot{H}}]}$$

and

$$K = 2.3 \times 10^{-9}.$$

If we further assume that the activity of the oxygen is directly proportional to its pressure, and put the activity equal to unity when the pressure is one atmosphere, then under constant pressure of oxygen the equilibrium conditions can be expressed as follows:

$$\frac{[A_{\ddot{Fe}}]}{[A_{\dddot{Fe}}]} [A_{\dot{H}}] = 2.3 \times 10^{-9} \qquad (F)$$

If, instead of assuming that the oxygen pressure is kept constant at the pressure of one atmosphere, we assume that it is kept constant at the pressure equivalent to its partial pressure in the atmosphere where there are 22 parts by volume of oxygen, we would then have,

$$[A_{O_2}]^{\frac{1}{4}} = (.21)^{\frac{1}{4}} = .677.$$

Under these conditions,

$$\frac{[A_{\ddot{Fe}}]}{[A_{\dddot{Fe}}]} [A_{\dot{H}}] = 3.4 \times 10^{-9}. \qquad (G)$$

If then a solution containing ferrous ions were oxidized reversibly by the atmosphere, equation (*G*) would express the equilibrium conditions. As a result of placing this solution in contact with solid ferric hydroxide and employing the calculation used in the former paper (11), equation (*G*) reduces to

$$\frac{[A_{\dot{\dot{F}}e}]}{[A_{\dot{H}}]^2} = 3.7 \times 10^{-3}. \qquad (H)$$

By multiplying both sides of this equation by the square of the dissociation constant of water one obtains the equation,

$$[A_{\dot{\dot{F}}e}]\,[A_{\bar{O}\bar{H}}]^2 = 3.7 \times 10^{-31}. \qquad (I)$$

We see then that this has led to the same general conclusion that was reached in the former paper; namely, that the oxidation has established the equivalent of a solubility product of ferrous hydroxide. The peculiarity of this solubility product is that if extra ferrous ions are introduced into this solution, they would not precipitate as ferrous hydroxide, but would be oxidized to the ferric state and be precipitated as the ferric hydroxide.

In a solution which was not undergoing such an oxidation Müller (18) determined the solubility product of ferrous hydroxide to be 1.64×10^{-14}, a value much larger than the one indicated by equation (*I*). The ultimate effect of such a reversible oxidation is then to reduce the solubility of the ferrous hydroxide by this enormous amount.

From equation (*I*) it is possible to see why a given amount of ferrous iron would be more completely oxidized under basic conditions than under acid conditions. Under acid conditions there could remain at equilibrium a relatively large amount of ferrous iron, whereas under basic conditions it would be almost completely removed from solution. In other words, under basic conditions ferrous iron is a much better reducing agent than under acid conditions. A mathematical demonstration of this fact has not been found in the literature, but in a qualitative way it has been observed that basic solutions of ferrous salts convert to the ferric form very rapidly, whereas acid solutions are oxidized by the oxygen of the air very incompletely and slowly (6, 9). Müller attempted to show this qualitatively by measuring the reducing power of ferrous solutions at various pH values by a direct measurement of the oxidation-reduction potential. In this study he showed that basic solutions of ferrous salts had a very high reducing value, whereas in acid solutions it was materially lower.

In the foregoing considerations it has been assumed that the oxidations by molecular oxygen were reversible. Under many conditions this may not be true. G. N. Lewis (14) found that he could not obtain the theoretical value of 1.227 in an oxygen-hydrogen cell, but by actual measurement found a lower value. Furman (8) by actual measurement found the e.m.f. of an oxygen-hydrogen cell to be 1.08 instead of the theoretical value of 1.227 as calculated

by Lewis. Montillon and Cassel (17) obtained the value of 0.99. These low values are undoubtedly due to the fact that in such a cell the oxidation is not entirely reversible. Under these conditions if we used the value $E_{o(O_2 - H_2O)} = 0.99$ in the foregoing calculations, we would obtain the value of

$$[A_{\dot{F}\dot{e}}] [A_{O\bar{H}}]^2 = 2.8 \times 10^{-27}. \qquad (J)$$

Under such conditions then, the oxidation would not be as complete as that indicated above.

It is to be expected, therefore, that if ferrous solutions were left to undergo atmospheric oxidation, this oxidation might be even more incomplete. This has been shown to be the case in a previous publication (11). It was found that if inorganic solutions were allowed to come to an apparent equilibrium by means of atmospheric oxidation the following relations seemed to exist:

$$\frac{[A_{\dot{F}\dot{e}}]}{[A_{\dot{H}}]^2} = 4.77 \times 10^3 \qquad (K)$$

and

$$[A_{\dot{F}\dot{e}}] [A_{O\bar{H}}]^2 = 4.77 \times 10^{-25}. \qquad (L)$$

Under these conditions the oxidation is even less complete than that indicated under equation (*J*). We can also see from this that if a solution that had undergone this natural oxidation would be made to undergo a reversible oxidation, the solubility of the ferrous hydroxide would decrease from a value indicated by the product 4.77×10^{-25} to a value 3.5×10^{-31}. This fact may be of some importance in connection with the physiology of the iron bacteria. It will also help to explain the precipitation of iron by some of the heterotrophic bacteria.

All of the aforementioned considerations have been with inorganic iron solutions. In organic solutions other factors enter that may increase the amount of iron that can remain in solution. Complex ions of iron may be formed which may reduce the ionic concentrations of either the ferrous or the ferric iron to extremely low values so that in many cases no precipitation of the ferric hydroxide will occur. This may also change the oxidation-reduction potential.

Since iron in nature is so frequently transported in the form of the carbonate, it is also of importance to inquire into the chemistry of iron solutions where carbonate may play an important rôle. It will be of interest, therefore, to find the equilibrium conditions in a solution where the following conditions will be fulfilled:

The solution is saturated with respect to CO_2.

The ferrous iron is being oxidized by molecular oxygen reversibly and exists in equilibrium as indicated by equation (J).

The gaseous phase in equilibrium with the liquid contains the same partial pressure of oxygen as that found in air. Not much error will be introduced by assuming that

when a small amount of CO_2 is introduced to the gaseous phase, the partial pressure of the oxygen remains unchanged since its influence upon the equilibrium is the quarter power of its activity.

The temperature of the system is kept at 25°C.

The solution is saturated with respect to ferrous carbonate.

In this solution then, the amount of CO_2 dissolved is determined by the partial pressure of this gas. According to the data of Bohr (2), at 25° when the CO_2 is under a pressure of one atmosphere, pure water in contact with this gas will dissolve in it 0.759 cc. of the gas per cubic centimeter measured at 760 mm., pressure at 0°C. From this we can calculate the relationship between the partial pressure of the gas and its concentration in the solution, if we assume that Henry's law holds. Henry's law must be taken to mean that when the pressure of the gas is doubled, it doubles the concentration of the unionized portion of gas dissolved in the water.

If d = density of the gas at 0°C. and M = molecular weight of the gas, then $\frac{0.759 \times d \times 1{,}000}{M}$ must be equal to the sum of the concentrations of CO_2, H_2CO_3, $H\bar{C}O_3$, and $\bar{C}\bar{O}_3$. When the partial pressure of the CO_2 on the water is one atmosphere,

$$\frac{0.759 \cdot d \cdot 1{,}000}{M} - \text{moles of ionized portion} = \text{moles of unionized portion.}$$

Since in the determination of the dissociation constants of carbonic acid, the unionized portion is represented as H_2CO_3, the same condition is assumed in the following calculations. Then at P atmospheres of pressure of CO_2 we have

$$\frac{P \cdot 0.759 \cdot d \cdot 1{,}000}{M} - \text{moles of ionized portion} = [H_2CO_3]$$

or

$$0.0339\ P - \text{moles of ionized portion} = [H_2CO_3].$$

It will be necessary, then, to find out what portion of the 0.0339 moles of the CO_2 dissolved in free water at 25°C. are unionized. This can be calculated from the following equation:

$$H_2CO_3 \rightleftarrows \dot{H} + H\bar{C}O_3$$

There will be $0.0339 - X$ moles of H_2CO_3, X moles of $\dot{H}$, and X moles of $H\bar{C}O_3$. From the mass law we can obtain an equation where X is the only unknown; namely,

$$\frac{X^2}{0.0339 - X} = K = 3.47 \times 10^{-7}. \quad (12)$$

From this equation X has the value of 0.00002. This is so small that it can be neglected. Therefore, we write the equation

$$0.0339\ P = [H_2CO_3]. \qquad (M)$$

From this equation the relationship between the partial pressure of the CO_2 and the concentration of the hydrogen and $\overline{\overline{CO}}_3$ ions can be found.

$$H_2CO_3 = 2\dot{H} + \overline{\overline{CO}}_3$$

$$\frac{[\dot{H}]^2\,[\overline{\overline{CO}}_3]}{[H_2CO_3]} = K = 1.80 \times 10^{-17} \quad (12)$$

or,

$$[H_2CO_3] = \frac{[\dot{H}]^2 \cdot [\overline{\overline{CO}}_3]}{1.80 \times 10^{-17}}.$$

When this value of $[H_2CO_3]$ is put in equation (M) the following results:

$$P = [\dot{H}]^2 \cdot [\overline{\overline{CO}}_3] \cdot \frac{10^{17}}{1.80 \times .0339}$$

$$P = 1.64 \times 10^{18}\,[\dot{H}]^2\,[\overline{\overline{CO}}_3]. \qquad (N)$$

In the solution described in the foregoing, one of the restrictions was that it should be saturated with ferrous carbonate. From the solubility product of this compound,

$$[\ddot{Fe}]\,[\overline{\overline{CO}}_3] = K = 3.45 \times 10^{-11}.$$

Putting this value in equation (N), we obtain the following,

$$P = \frac{1.64 \times 10^{18}\,[\dot{H}]^2\,3.45 \times 10^{-11}}{[\ddot{Fe}]} = 5.65 \times 10^{7}\,\frac{[\dot{H}]^2}{[\ddot{Fe}]}. \qquad (O)$$

Another restriction on this solution was that it should be in a state of equilibrium as indicated by equation (I) viz.,

$$[\ddot{Fe}] \times [\bar{O}H]^2 = 3.7 \times 10^{-21}.$$

By dividing both sides of this equation by the square of the dissociation constant of water, we get

$$\frac{[\ddot{Fe}]}{[\dot{H}]^2} = 3.7 \times 10^{-3}.$$

When this is substituted in equation (*O*),

$$P = \frac{5.67 \times 10^{7}}{3.7 \times 10^{3}} = 1.54 \times 10^{10}.$$

In other words, such a solution is non-variant, as far as the pressure is concerned, at a constant temperature. It can exist only at a partial pressure of CO_2 of 1.54×10^{10} atmospheres, a value which is probably never attained.

If it is assumed that an oxidation-reduction potential exists, as was found in the solution of ferrous sulfate in which the ratio of the ferrous ions to the hydrogen ions squared was 4.77×10^{3}, the equilibrium pressure would be

$$P = \frac{5.67 \times 10^{7}}{4.77 \times 10^{-3}} = 1.17 \times 10^{4}$$

Even this value is very large.

Let us assume that the solution is maintained at a very low oxidation-reduction potential, but reversible however, so low in fact that the product $[Fe][OH]^2$ approaches the solubility product as a limit. In other respects the solution is not changed. In this case, in the limit where the solubility product is 1.64×10^{-14}, the ratio of the ferrous ions to the square of the hydrogen ions becomes 1.64×10^{14}, as found by Müller (18). In this case,

$$P = \frac{5.67 \times 10^{7}}{1.64 \times 10^{14}} = 3.45 \times 10^{-7}$$

The partial pressure of the atmospheric CO_2 is 3.0×10^{-4}. In this last solution then we have one that may exist under certain conditions in nature. This will be discussed later.

From the foregoing equations, and equilibrium pressures of CO_2, predictions can be made as to what will happen if CO_2 is added or taken away from the system. If a small amount of CO_2 should be removed from the system in an attempt to lower its partial pressure, some of the solid ferrous carbonate in contact with the solution would dissolve in order to increase the concentration of the carbonate ion that had been lowered by the removal of the CO_2. This in turn would increase the concentration of the ferrous ion above a stable value. This would be compensated for by some of these being oxidized to the ferric state and being precipitated as ferric hydroxide. If CO_2 were continually removed from the system, the change would continue until all of the solid ferrous carbonate had dissolved. After that, further removal of the CO_2 according to the equation would result in a lowering of its partial pressure. The actual amount of ferrous iron in solution at the time when all solid ferrous carbonate is spent would depend on the acidity of the solution at that time. It can be readily seen from the above equations that the pressure of the CO_2 when the above solutions are in equilibrium is independent of the acidity. After all the solid ferrous carbonate is spent, however, a removal of the CO_2

would be accompanied by a decrease in the acidity in accordance with the fall of the partial pressure of the CO_2, according to the equation:

$$H_2O + CO_2 = H_2CO_3 = 2\dot{H} + \ddot{CO_3}.$$

As the acidity of the solution decreases, a precipitation of iron would occur as ferric hydroxide in accordance with the value of the ratio of ferrous ions to the square of the hydrogen ions. The value of this ratio depends, of course, upon the magnitude of the oxidation-reduction potential, as has been shown. If CO_2 should be added to a system that is in equilibrium with solid ferrous carbonate and ferric hydroxide and to one where there is a reversible oxidation-reduction by the O_2, this addition would bring about first a slight increase in the acidity, which would allow some of the ferric hydroxide to dissolve, and be reduced to the ferrous form. This would in turn supersaturate the solution with ferrous carbonate. A precipitation of this would then result. If CO_2 were continually added, this change would continue until all of the ferric hydroxide were dissolved. After this, further addition of CO_2 would increase the acidity and the concentration of the carbonate ion.

If CO_2 is added to a solution that contains no ferrous carbonate but is in equilibrium with solid ferric hydroxide, and a reversible oxidation-reduction is operative, such addition would cause an increase in the acidity, which in turn would dissolve some ferric hydroxide. Some of this would be reduced to the ferrous form. Further additions of CO_2 would continue this process until the solution became saturated with respect to ferrous carbonate.

By examining the equations from which was derived the equilibrium pressure of CO_2 at the various oxidation-reduction potentials, it can be readily seen that the pH of the various solutions cannot be determined from those equations. It would appear as though the pH might be changed without effect upon the system. This cannot be the case. It will be shown later that the pH can be changed within limits but not indefinitely. When solutions that contain no other ions but those furnished by the ferrous carbonate and hydroxide come to an equilibrium as has been indicated, the pH of the solution must be fixed. The pH, like the pressure of the CO_2, must depend upon the oxidation-reduction potential. From the following set of equations, its value can be determined. Since the solution must be electrically neutral, the sum of the charges must be equal to zero.

$$[\dot{H}] + 2[\ddot{Fe}] - 2[\ddot{CO_3}] - [OH^-] = 0 \qquad (a)$$

$$[\dot{H}][OH^-] = 10^{-14} \text{ (dissociation constant)} \qquad (b)$$

$$\frac{[\ddot{Fe}]}{[\dot{H}]^2} = K \text{ (from foregoing)} \qquad (c)$$

$$1.64 \times 10^{18} [\dot{H}]^2 [\overline{\overline{CO_3}}] = P_{CO_2} \text{ (from above)} \qquad (d)$$

When the oxidation potential is fixed and when a definite value is therefore placed upon the partial pressure of the CO_2, there remain then four unknowns, related by four independent equations from which all the variables may be determined. The most convenient way to solve this is to substitute in equation (*a*) for the ferrous, the carbonate, and the hydroxyl ions their equivalent in hydrogen ions as determined by equations (*b*), (*c*), and (*d*). There is obtained then a cubical equation that can be solved most readily by graphical means. Once having found the value of the hydrogen-ion concentration one can obtain the others readily from equations (*b*), (*c*), and (*d*). In table 1 are given the equilibrium values of the hydrogen-, the ferrous-, and the carbonate-ion concentrations calculated in this manner for the different states of oxidation discussed in the foregoing.

TABLE 1

Equilibrium values of the hydrogen-, Ferrous- and carbonate- ion concentration

NUMBER	$\frac{Fe^{\cdot\cdot}}{H^{\cdot 2}}$	P_{CO_2}	pH	H^+	$Fe^{\cdot\cdot}$	CO_3^{--}	$Fe(HCO_3)_2$	TOTAL Fe
1	3.5×10^{-3}	1.54×10^{10}	2.57	2.71×10^{-3}	2.56×10^{-3}	1.28×10^{-3}	4.9×10^{2}	490
2	4.77×10^{3}	1.17×10^{4}	4.61	2.45×10^{-5}	2.86×10^{-6}	1.19×10^{-5}	3.4×10^{-3}	3.4×10^{-3}
3	1.64×10^{14}	3.45×10^{-7}	9.50	3.16×10^{-10}	1.63×10^{-5}	2.11×10^{-6}	1.0×10^{-14}	1.63×10^{-5}
4	4.77×10^{3}	3.00×10^{-4}	6.15	6.89×10^{-7}	2.26×10^{-9}	3.36×10^{-7}	1.6×10^{-13}	2.26×10^{-9}
5	3.5×10^{-3}	3.00×10^{-4}	6.15	6.89×10^{-7}	1.66×10^{-14}	3.36×10^{-7}	1.2×10^{-13}	1.67×10^{-14}

In table 1 the amount of the unionized ferrous bicarbonate was determined as follows: From equation (*M*) the unionized carbonic acid is related to the pressure of the carbon dioxide as follows:

$$P \cdot .0339 = [H_2CO_3]$$

At a definite pressure then, from this equation, it is possible to calculate the CO_2 in the solution. From the table given by Smith (21) it is possible to find the amount of ferrous bicarbonate in solution. In order to get values for the pressure above and below those given in Smith's data, a straight line relationship was assumed to exist between the dissolved CO_2 and the concentration of the ferrous bicarbonate. The amount of ferrous bicarbonate was then obtained from this relationship.

The values in this table are not to be looked upon as accurate values from which quantitative deductions can be drawn. The various assumptions that have to be made in deducing these values introduce errors in the more concentrated solutions, that is, solutions which are under one or more atmospheres of CO_2. In the dilute solutions, they will be more accurate. The values given are to serve rather as indicators, in a qualitative way, of the types of changes that may take place under various conditions.

By making use of the equations and calculated values expressing the various equilibrium conditions, developed in the foregoing, it will be possible to draw conclusions regarding the following points:

Conditions which will favor the iron carrying capacity of water.
Conditions under which iron will precipitate in nature due to purely chemical reactions, in the absence of microbial activity.
Conditions under which microbial activity may be responsible directly or indirectly for the solution of iron and its reduction from the ferric to the ferrous form.
Conditions under which iron may be precipitated in nature by microörganisms.
Conditions under which microbial activity may bring about the precipitation of ferrous carbonate.

CONDITIONS WHICH WILL FAVOR THE IRON CARRYING CAPACITY OF WATER

It can readily be seen from table 1 on the equilibrium states in ferrous carbonate solutions that as the acidity increases, the iron content also increases. In nature about the only way in which the acidity can be increased to any great extent in natural waters is by the addition of CO_2. This naturally makes the acidity of the water dependent upon the properties of carbonic acid. By examining the equation,

$$\frac{[\ddot{Fe}]}{[\dot{H}]^2} = K,$$

it would appear that the larger this ratio the more iron will be held in solution. This would be strictly true if the acid that furnished the hydrogen ions were a strong acid and if the salt of iron formed by this acid be very soluble. This is not true with carbonic acid, however. Because ferrous carbonate has a rather low solubility product, because the carbonic acid is a weak acid, and because the bicarbonate of iron is readily soluble, it happens that the most favorable conditions for maintaining a high iron content in the water is to have a comparatively low value for the ratio of ferrous ions to the square of the hydrogen ions. Under these conditions only will the concentration of the ferrous ions in the solution be suppressed low enough to allow a sufficiently high carbonate-ion concentration to maintain an acid solution. When the aforementioned ratio is high it permits such a high ferrous-ion content in the solution that any attempt to increase the carbonate-ion content of the solution by increasing the pressure of CO_2 will result only in a precipitation of ferrous carbonate. Thus we see from table 1 that when the oxidation-reduction potential is high as indicated by an equilibrium ratio of

$$\frac{[\ddot{Fe}]}{[\dot{H}]^2} = 3.5 \times 10^{-3},$$

it is possible to increase the CO_2 pressure on the system to the very high pressure of 1.54×10^{10} atmospheres before any precipitation of ferrous carbonate

would take place, thus making it possible to increase the acidity to a point where a large amount of iron can be held in the solution. It is further seen that when the oxidation-reduction potential reaches the very low value where it permits the high stable ratio of

$$\frac{[\ddot{Fe}]}{[\dot{H}]^2} = 1.64 \times 10^{14},$$

the pressure of CO_2 cannot be increased above the low value of 3.45×10^{-7} atmospheres before a precipitation of ferrous carbonate would take place. This would make it impossible under these conditions to increase the acidity high enough with carbonic acid to allow the solution to hold more than a mere trace of iron. It is to be noted, however, that at a definite pressure of CO_2 it is possible to increase the iron content of the solution by decreasing the oxidation-reduction potential to an optimum value. Thus we see that when the CO_2 pressure is 3.00×10^{-4} atmospheres, more iron is held in the solution when the stable ratio of

$$\frac{[\ddot{Fe}]}{[\dot{H}]^2} = 4.77 \times 10^{3},$$

that when this ratio is forced to the lower value of 3.5×10^{-3}. If this ratio could be increased above the former value, which would be equivalent to decreasing the oxidation-reduction potential, it would soon increase the ferrous-ion concentration to a point where the ferrous carbonate would precipitate. Decreasing the oxidation potential still more would not further increase the concentration of the ferrous ion. This permits the rather definite conclusion that in systems where there exists a reversible oxidation-reduction for a given pressure of CO_2, there is a definite oxidation-reduction potential which will allow the maximum amount of iron to remain in solution. If the oxidation-reduction potential is changed in either direction from this value the iron content will be decreased. If the oxidation-reduction potential is increased, ferric hydrate will precipitate, and if it is decreased, ferrous carbonate will precipitate.

CONDITIONS FAVORING THE PRECIPITATION OF FERRIC HYDRATE FROM CARBONATED WATER IN THE ABSENCE OF MICROBIAL ACTIVITY

From the equations developed it can be readily seen that ferric hydrate in natural waters can be precipitated either by coming in contact with an atmosphere with a lower pressure of CO_2 than the CO_2 pressure of the solution; or by coming in contact with an environment of higher O_2 pressure, which in turn would increase the oxidation-reduction potential of the solution; or by undergoing both of these changes. Under natural conditions where iron is transported as the bicarbonate in underground waters it is difficult to estimate what

the actual oxidation-reduction potential might be. By examining the table 1 representing the equilibrium values, it can be seen that when any solution that is in equilibrium with ferrous carbonate and ferric hydroxide comes to the surface where it will be subjected to the atmospheric pressure of CO_2 and O_2, a large percentage of the iron carried by the water will be precipitated as ferric hydrate. Thus we see that solutions 1 and 2, which have an equilibrium pressure of CO_2 greater than the CO_2 pressure of the air, lose most of their iron when they finally reach the state indicated by solution 4. The oxidation-reduction potential of solution 1 is greater than that produced by the oxygen of the air, while the corresponding potential of solution 2 is the same as that produced by the air. Since in both of these solutions there is no increase in the oxidation-reduction potential as they pass from state 1 or 2 to 4, it must be concluded that the only cause for the precipitation here is the loss of CO_2 from the solutions as they pass from state 1 or 2 to 4. It is easy to conceive how this change would take place. As the CO_2 escapes from these solutions the acidity naturally decreases. This decrease in the H makes it necessary for the $Fe^{\cdot\cdot}$ iron to be oxidized to the ferric state and to be precipitated as the ferric hydroxide in order to maintain the stable ratio of $\frac{[Fe^{\cdot\cdot}]}{[H]^2}$ allowed by the oxidation-reduction potential of the solution. We see that in both of these solutions practically 100 per cent of the iron can be precipitated without the aid of any microorganism. It is not likely that the precipitation would entirely cease when it reached the state indicated by solution 4, when only 2.26×10^{-9} moles per liter of iron remain per liter, but any oxidation and precipitation taking place beyond this point would be so slow as to be of no importance in natural transformations. It is likely that the precipitation would be very slow before the concentration of the ferrous iron reached the value of 2.26×10^{-9} moles per liter. Theoretically the change should continue to state 5 with the very low value of iron concentration of 1.67×10^{-14} moles per liter. The fact that the oxidation of this type by molecular oxygen is not entirely reversible will prevent natural waters from reaching this very low value of iron content by pure chemical reactions in the absence of microbial activity.

In case of solution 3, when it changes to state 4 and decreases its iron content from 1.63×10^{-5} to 2.26×10^{-9} moles per liter, the cause of the precipitation is not the loss of CO_2. In this case the CO_2 tension of the liquid is less than that of the air. There is a marked increase in the oxidation-reduction potential that takes place as the conditions are changed from state 3 to 4. In this case, then, the increased oxidation-reduction potential must be considered as the cause of the precipitation of the iron.

In many cases there may be underground waters existing that are in a state intermediate between those found in solutions 2 and 4. Under those conditions, when these waters come to the surface, there will not only be a decrease in the CO_2 pressure, but there will also be an increase in the oxidation-reduction potential. In such cases the precipitation would be caused by both the loss of CO_2 from the water and by the increased oxidation-reduction potential.

In all of these cases then, it can be seen that nearly 100 per cent of the iron can be precipitated as the ferric hydrate by pure chemical reaction without the aid of microorganisms.

It is also theoretically possible to effect the precipitation of the iron by an increase in the alkalinity of the water other than that caused by the loss of CO_2. This can be done by having the water come in contact with the alkaline material or be diluted by alkaline solutions. Under natural conditions one way in which this is likely to happen is when underground water containing iron comes into contact with soil or water containing bicarbonates of strong alkalies, such as sodium or calcium. When the acidity is changed in this way without changing the CO_2 tension of the solution or the oxidation-reduction potential, the precipitation is caused exclusively by the change of reaction. The new lower value of H will naturally bring about a decrease in the Fe content in order to maintain the stable ratio of $\frac{[Fe^{\cdot\cdot}]}{[H]^2}$ under those conditions. The ferrous iron content would be decreased by being oxidized to the ferric form and precipitated as the ferric hydroxide. Such precipitation may be responsible for certain of the "hard-pan" formations occurring in certain soils. These formations, in the light of the foregoing deductions, can easily be explained by the last mentioned method of iron precipitation resulting from underground iron-bearing water coming into contact with lime-bearing water or calcareous soil near the surface. Such precipitation may also follow microbial activities. These have been considered in a former paper on the effect of heterotrophic bacteria on iron precipitation (22).

CONDITIONS UNDER WHICH MICROBIAL ACTIVITY MAY BE RESPONSIBLE FOR THE SOLUTION OF IRON AND ITS REDUCTION FROM THE FERRIC TO THE FERROUS FORM

From the foregoing theoretical considerations it has been pointed out that the ratio of the ferrous-ion concentration to the square of the hydrogen-ion concentration is dependent upon the oxidation-reduction potential, provided that the oxidation is effected by molecular oxygen. The ratio increases with a decrease in the oxidation-reduction potential. An increase in acidity of the solution and a lowering of the oxidation-reduction potential should then favor the solution and reduction of the iron. In many cases the acidity may be due to carbonic acid. Too high concentrations of this acid may limit the amount of iron that can be held in solution, as has been pointed out. As long as the amount of carbonic acid is relatively small only the oxidation-reduction potential and the acidity need be considered.

Solutions in which bacteria are growing, even though they be under aerobic conditions, should, in most cases, favor the solution of ferric iron and its reduction to the ferrous form. This should be particularly true if the solution becomes more acid as a result of the bacterial growth. This phase of the problem was considered in detail in a former publication in which experiments

and data are given showing how microbial activity brings about the reduction of ferric iron to the ferrous form. The condition that favors the solution of iron from its salt and oxides should also favor the solution of free iron from the metallic state. Grant (10) found this to be the case in some instances in the corrosion of iron in the soil. He found that bacterial activity greatly increased the rate at which the iron disappeared.

TABLE 2

Effect of bacterial activity upon the dissolving action of liquids upon metallic iron

SOLUTION	pH	REDUCED IRON	TOTAL IRON
		mgm. per 100 cc.	*mgm. per 100 cc.*
Part A. Iron filings added before sterilization			
Dextrose uninoculated	3.46	Trace	Trace
Dextrose uninoculated	3.54	Trace	Trace
Dextrose inoculated	6.40	190	180
Dextrose inoculated	6.13	224	225
Peptone uninoculated	6.70	0	0
Peptone uninoculated	5.60	0	0
Peptone inoculated	5.40	16	18
Peptone inoculated	5.50	14	15
Part B. Iron filings after sterilization			
Dextrose uninoculated	5.66	Trace	Trace
Dextrose uninoculated	5.86	Trace	Trace
Dextrose inoculated	6.20	130	130
Dextrose inoculated	6.23	121	120
Peptone uninoculated	3.70	None	None
Peptone uninoculated	3.72	None	None
Peptone inoculated	5.50	10	10
Peptone inoculated	5.52	10	10
Part C. No iron filings added			
		IRON	
Dextrose uninoculated	6.00	None	...
Dextrose inoculated	2.40	None	...
Peptone uninoculated	6.70	None	...
Peptone inoculated	5.60	None	...

For this experiment media were prepared having the same composition as those used in the experiment on ferric hydroxide. To three flasks containing 1 per cent dextrose, iron filings were added, as was also done to three flasks containing 1 per cent peptone. To four flasks, one containing dextrose and one peptone, no iron was added. All the flasks were sterilized in the autoclave at 15 pounds steam pressure for 30 minutes. Two of the dextrose flasks containing iron filings and one containing no iron were inoculated with soil and incu-

bated under anaerobic conditions, as used in the previous experiments, for 3 weeks. A corresponding set of the peptone flasks were treated in the same way. All the others remained uninoculated as controls.

Since it was conceived possible that the high temperature used in sterilization might in itself dissolve a considerable portion of the iron, a similar set of flasks was prepared that was sterilized first and the iron filings then added prior to incubation. These flasks were inoculated in the same order as the foregoing. Good growth resulted in all the inoculated flasks. Subsequent to incubation, the solutions were analyzed for soluble iron and acidity by pH measurements. The results of this experiment are given in table 2.

Here also it can be seen that under conditions where bacteria are growing and forming anaerobic conditions, a considerable amount of iron is dissolved and held in solution in the reduced form. This was particularly striking in the dextrose cultures. Peptone cultures do not show true results; because in the breakdown of the peptone rather large amounts of H_2S are formed which precipitate the iron as the sulfide. The inoculated peptone cultures showed a large amount of black precipitate. Not a very striking difference occurs between the cultures where the iron was added before and after sterilization. In the sterile cultures a considerable amount of iron oxide had formed, but very minute quantities remained in solution. In the dextrose cultures that contained the large amounts of iron, a very rapid oxidation and precipitation occurred after the solutions were exposed to the atmosphere. This precipitation was so rapid that it was necessary to analyze the solutions as soon as the stoppers were removed. In two instances, because of an unavoidable delay, some precipitation had occurred before the sample for the total iron had been taken. This accounts for the low results in these two cases. After the solutions had remained open to the atmosphere not more than 5 minutes, an extremely heavy precipitate of ferric hydroxide had formed.

From the results of this experiment it is easy to see that bacterial activity may become a very important factor in soil corrosion. By the action of bacteria a large amount of iron can remain in the solution and thus favor the solution of the metallic iron (22). Furthermore, bacterial activity will prevent the formation on the surface of the iron of an oxide film which is regarded as a protective film that retards corrosion.

CONDITIONS PREDISPOSING THE PRECIPITATION OF IRON

The effects of the heterotrophic bacteria on the precipitation of iron by changing the environmental conditions have already been considered, in a former publication.

Bacteria may be concerned in the precipitation of that iron in nature which is carried in solution in underground waters as ferrous bicarbonate, when this water comes to the surface. According to equations that have been developed previously, it was shown that rather large amounts of iron could be held in solution in underground water when the oxidation-reduction potential was

low. It was also shown that a very small amount of this iron could remain in solution under aerobic conditions. This would tend to suggest that it is unnecessary to assume any microbial activity to account for the precipitation of iron from such waters when they come to the surface. Undoubtedly, practically all the iron would precipitate without microbial activity. The fact remains, however, that in all the so-called iron springs, the ferric hydrate is found in the sheaths or bands of certain bacteria. This has led some bacteriologists to the assumption (3) that specific iron bacteria must be able to carry out precipitation of iron that would not occur in their absence. The equilibrium conditions that have been developed show conclusively that where large amounts of iron are in solution as the ferrous bicarbonate, this assumption is erroneous.

In table 1 it was shown that ferrous bicarbonate solutions, upon coming to the surface to atmospheric environment, could undergo a more or less rapid transformation until the iron content was reduced to about 2.3×10^{-9} moles per liter. It was further shown that this could be further reduced to 1.67×10^{-14} moles per liter if the oxidation were made reversible at the atmospheric pressure of oxygen. If there are bacteria, then, that can, either by enzymes or by their vital activity, bring about reversible oxidations, such bacteria could reduce the iron content of water from the value of 2.3×10^{-9} moles per liter to the value of 1.67×10^{-14} moles per liter. It is not to be assumed that this is a transformation that could not occur spontaneously. It must be pointed out that the first value was obtained by actual experiment in determining the extent of oxidation by the oxygen of the air in a solution of ferrous sulfate. In these experiments, the solutions of ferrous sulfate were allowed to stand for 4 months. It cannot be assumed that the reactions had gone to completion at that time. The ultimate end point after an infinite time should be the value of 1.67×10^{-14}. Reduction of the iron content from the value of 2.3×10^{-9} to 1.67×10^{-14} would be extremely slow. Bacteria that can increase the oxidation-reduction potential may speed up the reaction and in this way be of considerable importance in iron precipitation in low iron-bearing waters. It is only reasonable to assume that bacteria that can increase the oxidation-reduction potential of a solution will be concerned with iron precipitations in solutions where oxidation and precipitation by the atmospheric oxygen may be fairly rapid. If they create an oxidation-reduction potential that is much greater in the immediate neighborhood of the bacterial cells than in the portions of the solution away from the cells, the chances of iron being oxidized in or near the cells are much greater than away from the cells. The result of this will be that practically all of this iron will be oxidized in or near these organisms even in solutions where the iron content is considerably above 2.3×10^{-9} moles per liter. Such bacteria, then, can be regarded as taking advantage of a chemical reaction that is occurring spontaneously. It would not be necessary to assume that such organisms make use of the energy liberated by this reaction. It is possible, however, that in some cases, this energy is utilized by the organ-

isms in their growth. In the case of the autotrophic iron bacteria, there must be actual dependence upon this reaction (3). These autotrophs can be regarded as heterotrophs that have been living in the environment of this reaction until they have finally degenerated to a point where they are actually dependent upon the oxidation of ferrous to ferric iron for their energy.

In this connection it will be of interest to inquire into the usual iron content of water in places where iron bacteria are found in nature. Analyses were made of waters from four springs in which an abundant development of iron bacteria was found. Samples were collected in 300-cc. Erlenmeyer flasks. These were completely filled and tightly stoppered so as not to allow any great change to occur before they reached the laboratory. Samples were taken as close as possible to the point where the water first became exposed to the air and also just below the point where any development of iron precipitating bacteria was visible. In one case, samples were collected intermediate between these two points and also at a point some distance below the last visible sign of iron precipitate. The acidity was determined by pH measurements made by means of a Leeds & Northrup type "K" potentiometer with a calomel half cell and Hildebrandt hydrogen electrodes.

The iron content was determined [*see* San Yin Wong (20)] by a colorimetric method by evaporating 25-cc. samples to which had been added 1 cc. of concentrated H_2SO_4 until the solution fumed with SO_3 vapors. A 1-cc. portion of a 10 per cent solution of potassium chlorate was then added and the resulting solution again heated until SO_3 fumes again appeared. A second portion of 0.1 cc. of potassium chlorate was then added to assure the complete breakdown of any organic matter which might be present. After again being heated until SO_3 fumes appeared, the samples were made up to a volume of 15 cc. and to this was added 10 cc. of a 10 per cent solution of potassium sulfocyanate. A 1-cc. sample of a standard iron solution containing .0001 gm. of iron per cubic centimeter was treated in the same way. By comparing the color of the standard with the unknowns in a Dubosk colorimeter the iron content of the various samples was determined. In some of the samples that had very small amounts of iron it was found necessary to use 50-cc. samples of the water for the determination of the iron. In all the determinations it was found that the red color remained apparently unchanged for at least 10 minutes. The results of these analyses are shown in table 3.

Spring "a" was a very large spring found on the shores of the St. Croix river. In this spring, sample 1 was obtained just as the water emerged to the surface. It is not likely that any of the iron had precipitated from the water before this sample had been taken. Sample 3 was taken at the point where the iron precipitating bacteria seemed to cease developing. Sample 2 was taken at a point intermediate between 1 and 3. Sample 4 was taken at a point where the water from the spring had traveled about one quarter mile. The point where sample 3 was taken was about midway between the points where samples 1 and 4 were taken. A considerable amount of organic matter, par-

ticularly dead leaves, had been deposited in the water between the points where samples 3 and 4 were taken. This may have interfered with further precipitation of iron.

Spring "b" was a somewhat smaller spring also found near the St. Croix River. In this case a sample could not be obtained before any iron had precipitated, as the water passed through horizontal crevices in the rocks in which some of the iron was precipitating. Sample 1 was taken just where the water emerged from the crevice. Sample 2 was taken at the point where further precipitation was not visible. Sample 3 was taken after the water had moved about 100 feet beyond where sample 2 was taken.

Spring "c," also emptying into the St. Croix River, and spring "d," emptying into the Mississippi River, were the same type of springs as "b," and the same difficulty in getting the first sample was experienced. In both these

TABLE 3

The iron content and pH of natural iron springs

SAMPLE	pH	Fe	LOG Fe	$\log \frac{[Fe^{\cdot\cdot}]}{[H^{\cdot}]^2}$	$\frac{[Fe^{\cdot\cdot}]}{[H^{\cdot}]^2}$
		moles per liter			
1 a	6.8	17.4×10^{-5}	6.24–10	9.8	7×10^{9}
2 a	5.0	2.28×10^{-5}	5.36–10	5.4	2×10^{5}
3 a	4.8	1.73×10^{-5}	5.24–10	4.8	7×10^{4}
4 a	5.5	1.00×10^{-5}	5.00–10	6.0	1×10^{6}
1 b	6.0	10.16×10^{-5}	6.00–10	8.0	1×10^{8}
2 b	4.7	1.18×10^{-5}	3.07–10	4.5	3×10^{4}
3 b	4.8	0.49×10^{-5}	4.69–10	4.3	2×10^{4}
1 c	5.5	9.88×10^{-5}	5.99–10	7.0	1×10^{7}
2 c	4.6	1.16×10^{-5}	5.06–10	4.0	1×10^{4}
1 d	5.2	4.10×10^{-5}	5.61–10	6.0	1×10^{6}
2 d	4.6	1.82×10^{-5}	5.25–10	4.5	3×10^{4}

springs the second sample was taken below the point where no further visible precipitation of iron occurred.

In table 3 the ratio $\frac{[Fe^{\cdot\cdot}]}{[H^{\cdot}]^2}$ was calculated from the value of the iron content and pH value found. It has been shown that this ratio can be regarded as a measure of the oxidation-reduction potential. In the table these values indicate what the oxidation-reduction potential would have to be in order that the solutions with the corresponding amounts of iron might be stable. Under atmospheric conditions the apparent stable value of $\frac{[Fe^{\cdot\cdot}]}{[H^{\cdot}]^2}$ was found to be 7.3×10^{8}. It is apparent that in the analysis in all the springs tested, the iron precipitated rather rapidly until the ratio $\frac{[Fe^{\cdot\cdot}]}{[H^{\cdot}]^2}$ approached a value near 10^{4}. There is a little discrepancy in the case of sample 4 a. This can be explained

on the basis of the organic matter present in the water and which would tend to prevent precipitation of iron, either because of the formation of complex ions or the development of heterotrophic bacteria that would tend to prevent precipitation of the iron (22).

It is apparent from the analyses that the iron precipitation was brought about by the increased oxidation-reduction potential effected by the atmospheric oxygen. The ferrous iron was oxidized to the ferric and this was precipitated as the hydroxide, leaving the carbonic acid, which increased the acidity. The escape of the CO_2 from the solution was apparently slower than the oxidation and precipitation of the iron. There is an indication from the table that after the rapid precipitation had ceased there is a gradual decrease in the acidity as the CO_2 escapes. This is accompanied by a slow oxidation of iron. Since the iron contents of these solutions are not reduced below a point that would be reached by pure chemical oxidation in the absence of microbial activity, no great importance can be attached to the bacteria that are associated with the precipitation of iron in iron springs. It may be that the iron is oxidized faster in or near these bacteria but the fact that they do not decrease the iron content below a point where it would go in their absence speaks against the formation of any oxidizing enzymes that have been attributed to these organisms. These organisms can be regarded as profiting from a chemical reaction that would reach the same end point in their absence.

CONDITIONS WHICH WILL FAVOR THE PRECIPITATION OF FERROUS CARBONATE

It has been shown, in considering the system (CO_2, H_2O, ferrous and ferric iron), that, under certain conditions, a precipitation of ferrous carbonate may occur. Conditions which would favor such precipitations were found to be a low oxidation-reduction potential with a CO_2 pressure above the corresponding equilibrium pressure. It has been shown that when the oxidation-reduction potential is forced to a very low value, the corresponding equilibrium pressure of CO_2 is rather low.

It is not unreasonable to expect, therefore, that microorganisms may effect such precipitation. Heterotrophic bacteria creating anaerobic or semianaerobic conditions in environments where they produce rather large quantities of CO_2 should favor the precipitation of ferrous carbonate in media in contact with iron or its oxides or salts. Tache (24) found that, in bogs where a high bacterial development was taking place, ferrous carbonate accumulated in the anaerobic portions. Van Hise (25) shows that large deposits of ferrous carbonate, which he assumes are formed under conditions of low oxygen tension, are found in nature. In a series of flasks filled with a medium containing dextrose, 1 per cent; NH_4NO_3, 0.05 per cent; $MgSO_4 \cdot 7H_2O$, 0.01 per cent; and iron filings, and another series containing the same ingredients with the exception that 1 per cent peptone was substituted for the dextrose, it was found that a white precipitate formed in the bottom of those flasks which were inoculated

with soil and incubated under anaerobic conditions. This material effervesced when treated with HCl. Since the calcium and magnesium content of this medium was very low there remained only the iron to account for this carbonate. The precipitate was therefore considered to be ferrous carbonate. Because of the rapid decomposition and oxidation of this material when the flasks were opened to the atmosphere, no attempt was made to analyze the precipitate quantitatively.

CONDITIONS THAT MUST BE FULFILLED FOR THE CULTIVATION OF THE IRON BACTERIA

A few bacteriologists have reported that they have cultivated under laboratory conditions bacteria that depend for their energy upon the oxidation of ferrous iron to ferric iron. Notable among these are Winogradsky (26, 27) and Lieske (15). Others have reported that they could not get a development of these organisms. The work of Suessenguth (23) is of interest in this connection. He reports that he could not confirm the results of Lieske. Since these organisms must be regarded as being dependent upon a chemical reaction that is occurring spontaneously, it is possible to point out the conditions that must be fulfilled in order to develop these organisms under laboratory conditions.

In any medium where these bacteria are to be grown, the ferrous iron content must be maintained at a concentration where it is undergoing atmospheric oxidation. This means that in order to maintain growth, a natural iron spring has to be imitated or fresh amounts of ferrous carbonate have to be added to the solution continuously. These requirements are not easily carried out under conditions where pure cultures have to be maintained. I have tried both of these methods of cultivating iron bacteria, but in no case could an active culture be maintained. In every case the solutions became noticeably acid, so that the lack of bacterial development could be explained on the basis of high acidity. An abundant precipitate was obtained in every case with or without the inoculation of the media with material from natural iron springs.

Lieske (15) and Bass-Becking (1) have reported that they could cultivate these bacteria by suspending iron filings on the surface of synthetic media containing no organic matter. It is of course possible that metallic iron can supply enough dissolved iron to make conditions favorable for their growth. If this is to be done, ideal conditions for the slow oxidation of iron by free atmospheric oxidation must be maintained. The iron used will have to be free from all impurities that tend to catalyze the oxidation of ferrous iron to the ferric state. Otherwise there will result only an accumulation of rust on or near the filings and no or too few ferrous ions will be furnished to the medium to support growth. In this connection it is of interest to note that Bass-Becking (1) reports that small amounts of copper as an impurity in the iron filings interfered with the development of the bacteria. Maquenne (16) found

that copper catalyzes the oxidation of ferrous iron to ferric iron. This may account for the poisonous effect of the copper. Many attempts were made by the author to cultivate the so-called iron bacteria, employing the medium advocated by Lieske (15) and Bass-Becking (1), but in no case were successful cultures obtained. In these attempts both impure iron filings and filings prepared from the purest iron obtainable were used. In spite of all precautions, there must have been chemical catalyzers present that brought about an oxidation that was too rapid. Lieske states that the iron bacteria appear in about 4 days. In all of the author's attempts a heavy precipitate of ferric hydroxide appeared in 1 and 2 days. In 4 days the process seemed to be practically complete. No indication of bands of iron could be detected microscopically in the accumulated precipitate. In stained preparations many structures appeared that looked like bacteria and that retained the violet dye of the gram stain. Since these same structures were found by preparing a slide of iron filings it was concluded that these were not bacteria but only iron filings that stuck to the slide. It does not seem possible to maintain the autotrophic iron bacteria in pure culture with sufficient degree of certainty to allow extensive studies of their physiology.

It appears that the definite conclusion can be drawn that, in order to cultivate autotrophic iron bacteria, it is necessary to create such conditions in a medium where iron is continuously undergoing spontaneous oxidation from the ferrous to the ferric state that the bacteria in question can live by chance upon this reaction as a source of their energy.

SUMMARY

Equilibrium conditions have been considered in iron solutions under the influence of atmospheric oxygen and carbon dioxide, and equations have been developed expressing the equilibrium conditions.

Some of the activities of microorganisms associated with solution and precipitation as well as oxidation and reduction of iron are considered in the light of equations developed. The following observations were noted.

Under anaerobic conditions in dextrose or peptone media, heterotrophic microörganisms may dissolve metallic iron. They will also dissolve and reduce iron present as ferric hydroxide. These changes result from a decrease in the oxygen pressure and the formation of acid. These transformations may occur even at reactions close to neutrality.

Precipitation of ferrous carbonate may result under anaerobic conditions when CO_2 is formed by the breakdown of organic compounds.

Activity of iron bacteria appears to occur only under environmental conditions favorable to spontaneous oxidation by chemical agencies.

The iron content of natural water in iron springs is not reduced by the iron bacteria to a concentration below which it will go by a pure chemical reaction, under identical conditions.

The solution and precipitation of iron in nature are seen to be associated with equilibrium conditions which depend upon the oxygen tension, carbon dioxide tension and acidity, and the presence of organic compounds. These conditions may be modified very extensively by bacterial activity. Careful analysis of the activities of the so-called iron bacteria indicates

that their contributions to transformations of iron are confined solely to its precipitation. It is of great importance to note that their activity appears to occur only under those conditions in which the iron precipitates spontaneously, that they do not carry on the reaction beyond the degree arrived at by spontaneous precipitation. On the other hand it has been shown that bacteria not recognized as iron bacteria greatly modify their environmental conditions so as to bring about either the solution or precipitation of iron. Their effect upon the solution of iron can hardly be overemphasized in that they can effect changes favoring solution which do not occur spontaneously. Although the deposition of the oxide is seen to occur readily as a spontaneous precipitation, the deposition of the carbonate in nature indicates that the heterotrophic bacteria may also be of major importance in this transformation.

It would appear then that the importance of the true iron bacteria has been overemphasized, whereas the importance of the heterotrophic bacteria in transformations of iron in nature has not been fully appreciated.

REFERENCES

(1) Bass-Becking, L., and Meehan, Wm. J. 1927 Iron organisms *Science* 66: 42.
(2) Bohr, Chr. 1889 *Wied. Ann.* 68: 504.
(3) Cholodny, N. 1926 Die Eisenbakterian, Gustav Fischer, Jena.
(4) Clark, W. M. 1923 Public health reports. Reprint No. 823, v 38: 443–455.
(5) Ellis, D. 1919 Iron Bacteria, Metheren and Co., London.
(6) Ennos, F. R. 1913 Oxidation of ferrous salts. *Proc. Cambridge Phil. Soc.* 17: 182.
(7) Förster, Friedrich 1915 Electrochemie Wässeriger Losungen, ed. 2. J. A. Barth, Leipzig.
(8) Furman, N. H. 1922 Some applications of the oxygen electrode. *Jour. Amer. Chem. Soc.* 44: 2685.
(9) Gaines, R. H. 1910 Bacterial activity as a corrosive influence in the soil. *Jour. Indus. and Engin. Chem.* 2: 135.
(10) Grant, R., Bate, E., and Meyers, N. H. 1921 Influence of fermetantion products of bacteria on corrosion in condensers. *Commonwealth Eng.* 8: 364–366.
(11) Halvorson, H. O., and Starkey, R. L. 1927 Studies on the transformations of iron in nature: I. Theoretical considerations. *Jour. Phys. Chem.* 31: 626–631.
(12) Kendall, James 1916 The ionization constant of carbonic acid. *Jour. Amer. Chem. Soc.* 38: 1480–1497.
(13) Kolthoff, I. M., and Furman, N. H. 1926 Potentiometric titrations. John Wiley and Sons, Inc., New York.
(14) Lewis, G. N., and Randall, M. 1914 Free energy of O_2, H_2, and oxides of H_2. *Jour. Amer. Chem. Soc.* 36: 1969–1993.
(15) Lieske, R. 1911 Beitrage zur Kenntnis der Physilogie von Spirophyllum ferrugineum Ellis, einen typische Eisenbakterien. *Jahrb. Wiss. Bot.* 49: 91–127.
(16) Maquenne, L., and Demoussy, E. 1920 Catalytic action of copper salts on the oxidation of ferrous compounds by air. *Compt. Rend. Acad. Sci.* (*Paris*) 171: 65–69.
(17) Montillon, G. H., and Cassel, N. S. 1924 Acidity of cobalt and nickel plating baths. Use of the oxygen electrode. *Trans. Amer. Elect. Soc.* 45: 259–272.
(18) Müller, Erick 1908 Uber Die Reduzierende und Oxydierende Kraft von Eisensalzen. *Ztschr. Elektro. chem.* 14: 76–78.
(19) Peters, R. 1898 Uber Oxydations-und Reduktionsketten und den Einfluss komplexer Ionen auf ihre elektromotorishce Kraft. *Ztschr. Phys. Chem.* 26: 193–236.
(20) San Yin Wong. 1923 Colorometric determination of iron and hemoglobin in the blood. *Jour. Biol. Chem.* 55: 421–425.

(21) SMITH, H. J. 1918 On the equilibrium in the system ferrous carbonate, CO_2, H_2O. *Jour. Amer. Chem. Soc.* 40: 879–893.

(22) STARKEY, R. L., AND HALVORSON, H. O. 1927 Studies on the transformations of iron in nature- II. Concerning the importance of microörganisms in the solution and precipitation of iron. *Soil Sci.* 24: 381–402.

(23) SUESSENGUTH, K. 1927 Zur Kenntnis der Eisenbakterien der Gallionella-Gruppe. *Centbl. Bakt.* (II) 69: 327–339.

(24) TACHE, BR. 1923 The occurrence of natural ferrous carbonate. *Chem. Ztg.* 47: 845.

(25) VAN HISE, C. R., AND LEITH, C. K. 1911 The geology of the Lake Superior region. *U. S. Geol. Survey*, p. 520.

(26) WINOGRADSKY, S. 1888 Ueber Eisenbakterien. *Bot. Ztg.* 46: 262–270.

(27) WINOGRADSKY, S. 1922 Eisenbakterien als Anorgozygdanten. *Centbl. Bakt.* (II) 57: 1–27.